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Article type : Symposium Paper



# Fundamental structural and functional properties of Aquaporin ion channels found across the kingdoms of life.

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Short title: Roles of aquaporin ion channels

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1440-1681.12900

## Abstract

Aquaporin (AQP) channels in the Major Intrinsic Protein (MIP) family are known to facilitate transmembrane water fluxes in prokaryotes and eukaryotes. Some classes of AQPs also conduct ions, glycerol, urea, $CO_2$, nitric oxide, and other small solutes. Ion channel activity has been demonstrated for mammalian AQPs 0, 1, 6, Drosophila big brain (BIB), soybean nodulin 26, and rockcress AtPIP2;1. More classes are likely to be discovered. Newly identified blockers are providing essential tools for establishing physiological roles of some of the AQP dual water and ion channels. For example, the arylsulfonamide AqB011 which selectively blocks the central ion pore of mammalian AQP1 has been shown to impair migration of HT29 colon cancer cells. Traditional herbal medicines are sources of selective AQP1 inhibitors that also slow cancer cell migration. The finding that plant AtPIP2;1 expressed in root epidermal cells mediates an ion conductance regulated by calcium and protons provided insight into molecular mechanisms of environmental stress responses. Expression of lens MIP (AQP0) is essential for maintaining the structure, integrity and transparency of the lens, and Drosophila BIB contributes to neurogenic signalling pathways to control the developmental fate of fly neuroblast cells; however, the ion channel roles remain to be defined for MIP and BIB. A broader portfolio of pharmacological agents is needed to investigate diverse AQP ion channel functions in situ. Understanding the dual water and ion channel roles of AQPs could inform the development of novel agents for rational interventions in diverse challenges from agriculture to human health.

**Key words:** aquaporin; arylsufonamide; cation channel; divalent cation; fluid homeostasis; major intrinsic protein; metastasis; traditional herbal medicine; volume regulation; water channel

## Introduction

Aquaporins (AQPs) are involved in many functions, including maintaining osmotic water homeostasis, cellular structure, and volume regulation; enabling fluid flow across barrier tissues; supporting metabolic demands, cellular migration, and more. Maintaining water homeostasis is vital to every living organism. AQPs allow the transport of water molecules through intrasubunit pores down osmotic or hydrostatic pressure gradients (1), and are found across diverse species in prokaryotes and eukaryotes (2, 3). Fifteen aquaporin genes have been identified in mammals (AQP0-AQP14) (3, 4). Plants express many different MIP channels. Hundreds of AQP loci detected from genomic analyses of higher plants are divided into five subfamilies (5, 6).

AQPs organize as tetrameric pores in cell membranes ***(Figure 1)***. Each subunit consists of six membrane spanning helices and five loops (A to E); the amino and carboxyl terminal domains reside on the cytosolic side (7). Loops B and E, on the intracellular and extracellular sides respectively, fold inward to span the membrane as short helices, each with a conserved asparagine-proline-alanine (NPA) signature motif (7). The classification of mammalian AQPs 3, 7, 9 and 10 as aquaglyceroporins reflects their ability to allow permeation of small uncharged molecules such as glycerol in addition to water (4).

As illustrated in ***Figure 2***, aquaporins -0, -1, and -6, *Drosophila* Big Brain, plant AtPIP2;1 and Nodulin-26 have been shown to have ion channel activity (8-13). In AQP1 and AtPIP2;1 channels, the central pore of the tetramer has been proposed as the pathway for cation conductance (9, 14, 15). Intrasubunit pores are thought to be pathways for ion transport in BIB, AQP0 and AQP6 channels (16-18). Evidence for intrasubunit ion pores in BIB comes from mutational studies in which the change of a conserved glutamic acid at position 71 to aspargine (E71N) in BIB diminished the ionic conductance, whereas the equivalent mutation in AQP1 E17N did not prevent ion channel activity, but blocked AQP1 water channel function (18). In AQP6, the key residues that affect ion channel properties are located in loop B, a domain typically associated with the intrasubunit pore (17, 19).

## 1. Functional roles of mammalian AQP1 ion channels

The concept of ion channel activity in an aquaporin has been most comprehensively studied in mammalian AQP1 in terms of structure-function relationships, pharmacology, and physiological relevance. The number of AQPs designated as aquaporin ion channels is likely continue to increase as the originally disputed concept (20) gains acceptance and lines of evidence accumulate (21). Ongoing research is addressing the roles of AQP1 and other ion channels in key health challenges such as cancer metastasis and brain oedema.

### *1.1 Ion conductances needed for cancer cell migration*

Malignant transformation in cancers is marked by increased proliferation, aberrant apoptosis, angiogenesis, migration and invasion, all processes that are influenced by ion channels (22-26). Other work has added aquaporin water and glycerol channels to the list of channels that contribute to cancer cell migration and metastasis (27-31).

The AQP1 ion channel, gated by cyclic GMP (32-34), mediates nonselective monovalent cation currents (e.g., $Na^+$, $K^+$, and $Cs^+$), but is not appreciably permeant to divalent cations or protons (32, 35). Tetrameric organization of subunits around a central pore is a theme noted for many types of ion channels (36, 37). Evidence from molecular dynamic modelling and site-directed mutagenesis have converged on the idea that in AQP1 the ion permeation pathway is the central pore at the 4-fold axis of symmetry (33, 38). The gating of the AQP1 ion channel depends on cyclic GMP (32) which is thought to interact with an arginine-rich region of loop D, opening the central pore to allow hydration and permeation of water and cations (38). The responsiveness of AQP1 ionic conductance to cGMP is modulated by tyrosine phosphorylation at position 253 in carboxyl terminal domain of human AQP1 (39), and protein-kinase-C mediated phosphorylation at threonine residues 157 and 239 (40). AQP1 ionic current activation is less efficient when two key residues aspartate (D237) and lysine (K243) in the carboxyl terminal domain are mutated (41), further indicating the C-terminal domain influences the efficacy of cGMP-mediated activation. Site-directed mutagenesis of a conserved pair of arginine residues in loop D decreases the efficacy of inhibition by the AQP1 ion channel blocker AqB011 (Kourghi et al., 2017, in preparation), confirming an earlier proposal that the compound interacts at the loop D gating region (42). AQP1-expressing HT29 colon

cancer cells treated with AqB011 show impaired cell migration at doses that match the dose-dependent block of the ion channel conductance, recorded from cloned human AQP1 channels expressed in Xenopus oocytes (43). AQP1 expression also is associated with tumour angiogenesis; AQP1 knockdown or inhibition correlates with reduced growth (44).

Various classes of channels and transporters have been identified as key components in cell migration. The $Na^+/H^+$ exchanger (NHE1) influences cell migration, proliferation and volume regulation (45-47). Inhibition of NHE1 leads to decreased motility of tumour cells (48, 49). $K^+$ and $Ca^{2+}$ channels influence cell migration by regulating cell volume and membrane potential (50). Blocking SK3 $K^+$ channels reduces cancer cell migration and metastatic potential (51). The epithelial sodium channel (ENaC) and acid sensitive ionic channel (ASIC) also are linked to cell migration and invasiveness (52, 53). Reduced levels of ASIC1 and ENaC expression inhibit the migration of glioblastoma cells (54). Piezo ion channels, sensitive to membrane tension, function to transduce mechanical stimuli in diverse species, and serve roles that include cell migration, sensory perception and homeostatic regulation, with links to cardiovascular disease and cancer (55-57) .

Multiple $Ca^{2+}$ signalling pathways influence cancer cell migration and proliferation (58-61). Transient receptor potential (TRP) channels enable the localized entry of $Ca^{2+}$ in lamellipodial leading edges; *TRPM7* knockdown abolished $Ca^{2+}$ flickers in fibroblasts and impaired chemotactic steering of cell migration (62). Stores-operated

$Ca^{2+}$ entry augments intracellular $Ca^{2+}$ via a calcium release-activated calcium channel (Orai1) and a stromal interaction molecule 1 $(STIM1)^{+}$ (63); block of the $Ca^{2+}$ entry pathway by SKF96365 and 2-APB (2-aminoethyl diphenylborinate) decreased cancer cell migration and proliferation in clear cell renal cell carcinoma (64).

Relative contributions of the diverse classes of channels and transporters to cancer cell migration are expected to depend on tumour cell type, patterns of gene expression, interacting proteins, environmental conditions, and levels of activation of intracellular signalling pathways. AQP1 interacts with signalling pathways including MAP kinase, protein kinase C, Wnt, PI3 kinase, and TGF-β (40, 65-67), and is modulated by protein-protein interactions with carbonic anhydrase (68) and others. AQP1 in bone marrow mesenchymal stem cells promotes migration through focal-adhesion kinase and PI3K/Akt signalling pathways (69). Knockdown of AQP1 in human endothelial and melanoma cell lines disrupts actin cytoskeletal organization, reduces levels of interacting proteins such as Lin-7, and impairs cell migration (67), outcomes expected to compromise metastasis and angiogenesis.

AQP1 is one of many ion channels that merits exploration as a therapeutic target to control migration and metastasis during the progression of certain types of cancers. Inhibition of AQP1 ion channels would be expected to have clinical potential selectively for the subset of cancer types in which upregulation of AQP1 serves a key role in metastasis and angiogenesis (70).

### *1.2 Ion and water flux in cerebrospinal fluid (CSF) production*

A principal role of choroid plexus is secretion of cerebrospinal fluid (CSF). CSF fills the brain ventricles and spinal canal, providing physical support and a specialized environment for transport of nutrients, peptides, and hormones throughout the CNS

(71, 72). The choroid plexus, lining the ventricles in the brain, is a layer of cuboidal epithelial cells that interfaces between the blood capillary system and ventricular space (73, 74). The mechanism of CSF secretion involves bulk ion movement from the blood to the ventricle across the choroid plexus through transcellular transporters (75). In choroid plexus, transporters and ion pumps are differentially located on apical and basal membranes to create an apico-basolateral polarity. AQP1 is highly expressed in choroid plexus, specifically on the apical side (76, 77). The major function of AQP1 in choroid plexus is thought to be facilitation of water movement from the basolateral to the apical side of the barrier, following the gradient created by active transport of sodium ions through pumps and exchangers (78-80).

A role for the cation channel function of AQP1 in modulating CSF flow rate in choroid plexus was proposed in 2006 (81), but selective pharmacological agents for AQP1 were not available at the time to confirm the findings. The AQP1 ion conductance in primary cultures of choroid plexus was activated by cGMP, and the rate of CSF production was stimulated by application of atrial natriuretic peptide (ANP) which binds to an endogenous guanylate cyclase receptor, producing cGMP (82). When AQP1 ion channels were blocked with a non-selective antagonist $Cd^{2+}$, CSF production was slowed, as measured in a transwell primary culture model (81). Further work is needed to evaluate whether the AQP1 ion channel function regulates CSF secretion in vivo.

AQP1 ion channels could also contribute to functional roles in other tissues, in processes such as angiogenesis (83), fluid transport in renal proximal tubule (14), red blood cell adaptation to stressors, cancer metastasis, glaucoma, brain oedema, and more.

## 2. Diverse properties of ion channel aquaporins

### *2.1 Mammalian Aquaporin 0*

AQP0, also known as lens MIP or MIP26, is the major protein component of isolated lens junctions (84, 85). AQP0 has been shown to function as a water channel when expressed exogenously in Xenopus oocytes (86, 87) and endogenously in membrane vesicles generated from freshly isolated preparations of mouse, frog and rabbit lens fibres (88-90). The primary function of AQP0 in the lens may be more than membrane water permeability alone, and could involve cell-cell adhesion of lens fibres or regulation of gap junction channels. AQP0 has the lowest water permeability of mammalian AQPs 1 to 5, with single channel water permeability about 1/40th that of AQP1 (86). AQP0 is required for maintaining the transparency of the ocular lens (91); humans and mice lacking AQP0 develop congenital cataracts (92).

When reconstituted in bilayers, AQP0 shows ion channel activity (10, 93-95) characterised by large single channel conductance, a slight anionic selectivity, and symmetrical voltage dependence (94). Bovine AQP0 is voltage- and pH-sensitive, and generally closed at neutral pH (94). AQP0 channel openings have two main conductance states with amplitudes of 380 and 160 pS in 100 mM KCl. The water channel activity of AQP0 is dependent upon pH and calcium (96), suggesting that regulatory mechanisms modulate both the water flux and the ion conductance properties of AQP0. Minimizing extracellular space in the lens by enabling fluid flow into lens fibres could reduce light scattering, and thus assist in maintaining optimal transparency (97). Maintaining optimal lens transparency could utilise both the water and ion channel functions of AQP0.

### *2.2 Mammalian Aquaporin 6*

Aquaporin-6 (AQP6) is an anion channel and water channel that is atypically activated rather than blocked by mercuric chloride ($HgCl_2$) (13), unlike AQP1 (98) and other AQPs. AQP6 assembles as a tetramer, possessing monomeric pores for water and anions, with a permeability series of $NO_3$>I>>Br>Cl>>F (99, 100). Interestingly, when expressed in *Xenopus* laevis oocytes, AQP6 channels show low water permeability; however, exposure to $HgCl_2$ at concentrations up to 300 µM stimulates AQP6 water permeability more than five-fold, and the ion conductance more than six-fold (99, 101). Two cysteine residues (C155 and C190) are important for the $HgCl_2$ gating of AQP6. The AQP6 water channel activity and anion conductance are reversibly potentiated by low pH, suggesting a mechanism of activation with some potential physiological relevance (99).

AQP6 is present in intercalated cells of the renal collecting duct in mammals (102, 103). In α-intercalated cells, AQP6 was found to be colocalized with $H^+$/ATPases in intracellular vesicles but not in plasma membrane (99), and was suggested to contribute to urinary acid secretion and acid/base regulation (104). Significant upregulation of AQP6 expression was observed in rats exposed to chronic alkalosis or water loading, but not chronic acidosis (105). Expression of AQP6 in rat gastrointestinal epithelium, near tight junctions and secretory granule membranes in rat parotid acinar cells, and in some ovarian cancers (106-108) has suggested other possible roles in tissues involving acid-base regulation, although the physiological significance of AQP6 in these systems is not yet fully elucidated.

AQP6 has been proposed to contribute a protective role in some types of viral pathologies based on data showing AQP6 expression levels were inversely correlated with susceptibility to viral infection in host cell lines. Molinas and colleagues introduced GFP-tagged AQP6 into mouse fibroblast cells that were infected with Hazara virus, as a model for Crimean–Congo haemorrhagic fever. Overexpression of AQP6 reduced the infectivity of Hazara virus; conversely, cells that were infected with Hazara virus showed altered cell morphology and a reduced level of AQP6 expression at both protein and mRNA levels (109). Understanding the mechanism linking the AQP6 channel to pathological outcomes could open new opportunities for modifying cellular vulnerability to pathologies caused by certain vectors such as Hazara virus.

### *2.3 Arabidopsis thaliana PIP2;1*

The plant aquaporin AtPIP2;1 is a plasma membrane protein highly expressed in *Arabidopsis* roots and stomata, and is involved in maintaining plant water homeostasis (110). Water transport studies conducted on proteoliposomes showed that the osmotic water permeability of AtPIP2;1 channels was impaired by divalent cations, with the highest inhibitory efficacies shown by $Ca^{2+}$, $Cd^{2+}$ and $Mn^{2}$. Protons also blocked the water flux, with half-maximal inhibition at pH 7.15 (111). Calcium plays an important role in signal transduction in plants, particularly under stress conditions (112).

AtPIP2;1 channels expressed in Xenopus laevis oocytes carry cation currents that are sensitive to block by divalent cations and pH (9), confirming this channel is another example of the expanding list of dual water and ion channel aquaporins. AtPIP2;1 ion currents are blocked by extracellular $Ca^{2+}$ and $Cd^{2+}$ (9). The biphasic dose-response curve plotted for the ionic conductance amplitude as a function of EGTA-buffered free $Ca^{2+}$ level had a component with an $IC_{50}$ value of 0.32 mM which corresponds with values reported for $Ca^{2+}$ block of non-selective cation channels in Arabidopsis root protoplasts. Low external pH inhibited the ionic conductance ($IC_{50}$ pH 6.8). These data suggest that the AtPIP2;1 might carry the cationic conductance described in roots and guard cells that is known to be important for plant responses to environmental conditions, but was not previously defined at the molecular level. The ionic current in AtPIP2;1-expressing oocytes cannot be explained as an indirect result of native oocyte channels activating in response to swelling, since the co-expression of another related channel (AtPIP1;2) with AtPIP2;1 did increase water permeability but did not confer an ionic conductance response in the same conditions. Furthermore, the mutation of glycine at position 103 to tryptophan (G103W) in AtPIP2;1 impaired both ion and water channel activity, demonstrating the cation permeation is intrinsic to the AtPIP2;1 channel (9). Based on patterns of expression, AtPIP2;1 ion channels might explain the coupled ion and water transport known to facilitate rapid volume responses for guard cell closing (113, 114), and hypo-osmotic turgor in plants in the absence of water potential differences (115).

Water permeability through membranes expressing AtPIP2;1 channels is regulated via phosphorylation (113, 116). Precedent for the regulation of aquaporin ion channels by phosphorylation has been established for mammalian AQP1, in which the phosphorylation of tyrosine 253 in the carboxyl terminal domain of AQP1 has

been shown to govern the responsiveness of the ion channel to cGMP (39). The probability of AQP1 being available to be gated as an ion channel is enhanced when the tyrosine phosphorylated state of the channel is favored via treatment with a tyrosine phosphatase inhibitor, bisperoxo-(1,10-phenanthroline)-oxovanadate-(V) (39). Similar intracellular cascades involving cyclic nucleotide signalling in roots and phosphorylation of plant AQP channels (117, 118) could be explored as mechanisms for controlling dual water and ion transport through AtPIP2;1.

### *2.4 Drosophila* Big Brain

The transmembrane protein Big Brain (BIB), encoded by a Drosophila neurogenic gene, is a member of the aquaporin channel family (119). During neurogenesis in the early development of Drosophila, a loss-of-function mutation of the big brain gene (*bib)* causes the overproduction of neuroblasts (120). In parallel with other neurogenic genes *Notch* and *Delta*, BIB is involved in the process of lateral inhibition, and its absence leads to a pathological phenotype involving overproduction of neuroblasts (119, 120).

When expressed in *Xenopus* oocytes, BIB functions as a monovalent cation channel activated by membrane pricking or pharmacological modulation of tyrosine kinase signalling pathways, but is not a water channel (11). The BIB ion channel is inhibited by insulin-like receptor activation of a tyrosine kinase pathway; and conversely is activated by a tyrosine kinase inhibitor, lavendustin A (11). Tyrosine phosphorylation at predicted consensus sites in the carboxyl terminal domain was validated by western blot (11). The BIB ion channel showed voltage-sensitive block by divalent cations such as $Ca^{2+}$ and $Ba^{2+}$, suggesting the divalent binding site is within the

electrical field. A glutamate residue in the first transmembrane domain Glu$^{71}$, a position that is highly conserved in the MIP family, was defined as essential for divalent cation binding in BIB (121). A possible role for the predicted depolarising effect of BIB activation, as one of the components of lateral inhibitory signalling remains to be tested in developing Drosophila in vivo.

## 3. Future directions

Acquiring a broad perspective on the functional roles and regulatory controls for aquaporin dual water and ion channels will be critical for understanding the spectrum of potentially important physiological roles that these channels might serve, as well as their potential value as targets in an impressively diverse array of applications. Aquaporin modulators have promise for future therapeutic interventions in clinical disorders including cancer metastasis, renal failure, and brain pathophysiology, as well as in enhancing agricultural productivity in challenging environments, managing vectors of transmitted diseases, and much more.

The development of selective blockers has been a long awaited milestone, and is now allowing the characterization of aquaporin functions in living cells. Block by AqB011 of the AQP1 ionic conductance demonstrated that the cation channel activity is a key component for HT29 cancer cell migration (***Figure 3***) (42). The continuing evaluation of traditional medicinal plants as sources of blockers of AQP1 has produced agents that selectively inhibit water permeability (such as bacopaside II), or that block both the water and ion channel pores (such as bacopaside I); these also are promising tools for controlling migration in the subset of cancers that rely on

AQP1 expression (122). Bacopaside II isolated from the traditional medicinal herb Bacopa monnieri blocks the migration of cancer cells measured in live-cell-imaging assays (***Figure 4***) without toxicity at effective doses (122).

Dual water and ion channel AQPs are being recognized as important for modulation of transmembrane fluid gradients, volume regulation, signal transduction, and adaptations to environmental factors for many different types of organisms. An exciting future might harness AQP blockers as novel agents for slowing cancer metastasis, to be incorporated as adjunct treatments during primary surgical, chemo, or radiotherapy procedures. However, the translational value of new AQP pharmacological agents for limiting cancer metastasis awaits validation from in vivo studies and clinical trials. Work in progress suggests that selective inhibitors of AQP1 ion channels might also be useful in protecting red blood cells from pathological changes in cell morphology in some disease conditions (Kourghi et al., 2017, in preparation). Uncovering the multifunctional roles of aquaporins offers new pathways for basic research discovery and translational advances, and compels a renewed appreciation of the diversity and complexity of aquaporins in essential processes of physiology and pathology across all forms of life.

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## Figure legends

**Figure 1**: Structural organization of the Aquaporin-1 channel subunit.

A: Crystal structure of the human AQP1 monomer. Highlighted in blue is the loop D domain, in red are the double arginine residues at positions 159 and 160 (R159+R160) located in the loop D domain. The double arginine site is proposed to be involved in binding of the agonist cGMP and antagonists AqB011 and bacopaside I (15, 42, 122). Cysteine at position 189 (cyan) is the mercury binding site (98). Tyrosine 186 (magenta) influences the binding of tetraethylammonium as a water pore blocker (123). Threonines 157 and 239 (yellow) are thought to be PKC regulatory sites (40). The amino terminal domain is highlighted in green.

B. Membrane topology diagram of AQP1 showing membrane spanning helices, connected by 5 loops (extracellular A, C and E; intracellular B and D). The Asn-Pro-Ala (NPA) signature motifs are located on loops B and E. Key regulatory sites (as summarized in (A)) are highlighted, and include tyrosine Y253 (black) which when phosphorylated enhances activation of AQP1 ion channel currents (39).

**Figure 2**: Models illustrating the two proposed schemes for ion and water channel permeation through AQP1, AtPIP2;1, BIB, AQP0 and AQP6 channels. For AQP1 and AtPIP2;1 channels (left), ions are thought to move through the central pore of the tetramer (9, 15, 124), and the major water transport is through individual monomeric pores (7, 125). For BIB, AQP 0 and AQP6 (right), ion and water permeation is proposed to occur through the individual intrasubunit pores, not the central pore (16-18). Big Brain channels have not been found to have appreciable water permeability under conditions tested (121).

**Figure 3**: AqB011 is a specific blocker of AQP1 ion channel and impairs cancer cell migration in HT29 cancer cells (42). AqB011 is predicted to interact with the loop D gating domain of the channel. Inset: View of the putative binding site for AqB011 in the tetrameric AQP1 channel, predicted from in silico docking. The ligand is thought to interact with two conserved arginine residues (hAQP1 R159 and R160) in the loop D gating domain, a site needed for cGMP-mediated activation of the ionic conductance.

**Figure 4**: Temporal sequence from a live cell-imaging assay of wound closure, illustrating the robust inhibition of cell motility caused by treatment of HT29 colon cancer cells with the AQP1 water pore blocker bacopaside II (15 μM). Circular wounds were created in confluent non-proliferating monolayers of HT29 cancer cells. Single cells at the edge of the wounds were tracked in time lapse images over 24 hour periods, with and without bacopaside II. For clarity, only images at 8 hour intervals are shown. White arrows identify a single cell in each treatment at 0 hours, followed through subsequent frames.

**Figure 5:** Amino acid sequence alignment of loop D regions for BIB, hAQP1, AtPIP2;1 AtPIP2;2 and AtPIP2;7. After a conserved aspartate (D) residue, these selected channels show at least two positively charged amino acids (K lysine, or R arginine) in proximity. AQP1 lacks the adjacent proline (P), and instead has four arginines in series. The first two arginines (R159 and R160 in hAQP1) are implicated in ion channel gating and block by AqB011. Pharmacology for BIB, AtPIP2;1 AtPIP2;2 and AtPIP2;7 channels remains to be defined.

**Figure 1**

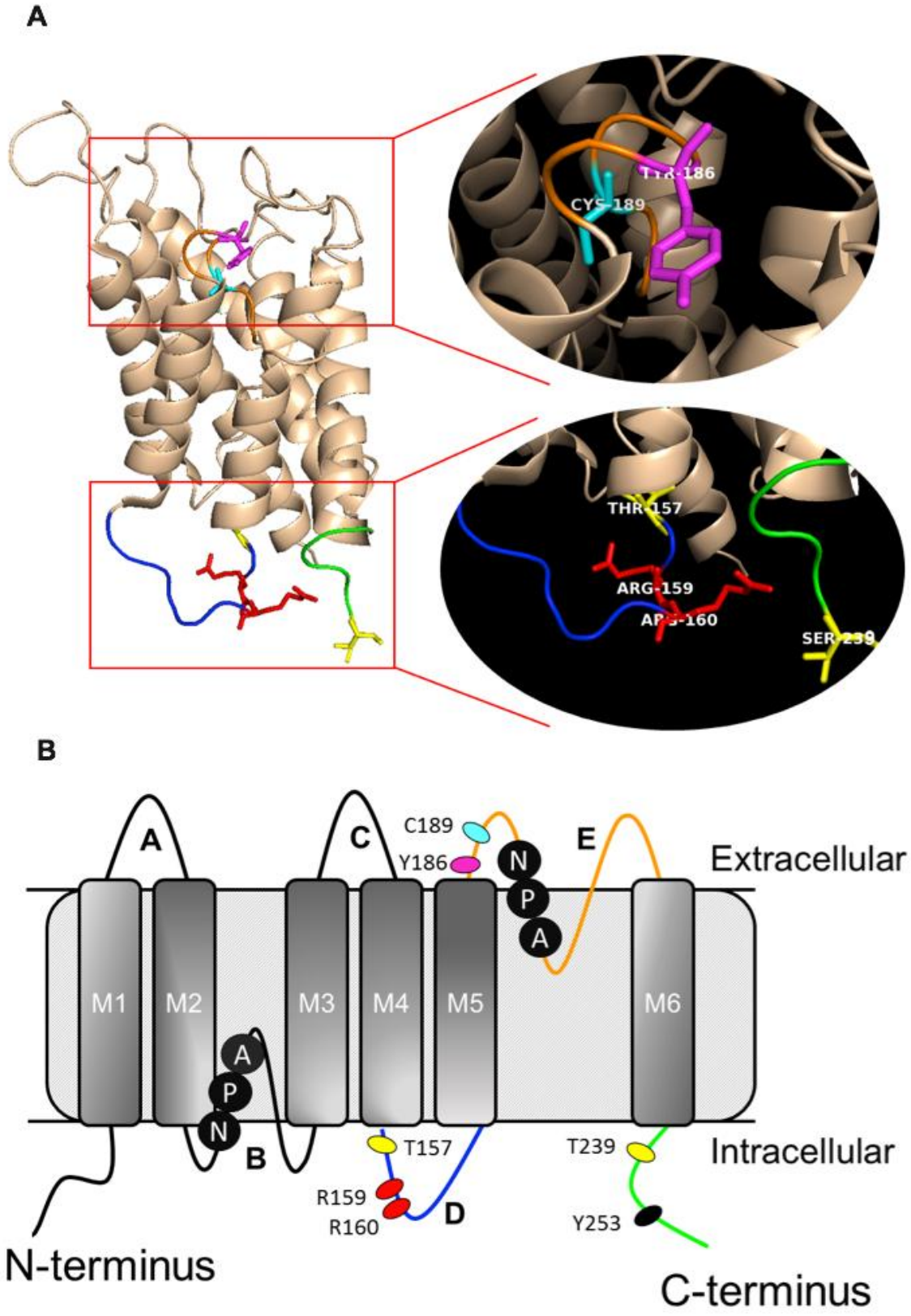

**Figure 2**

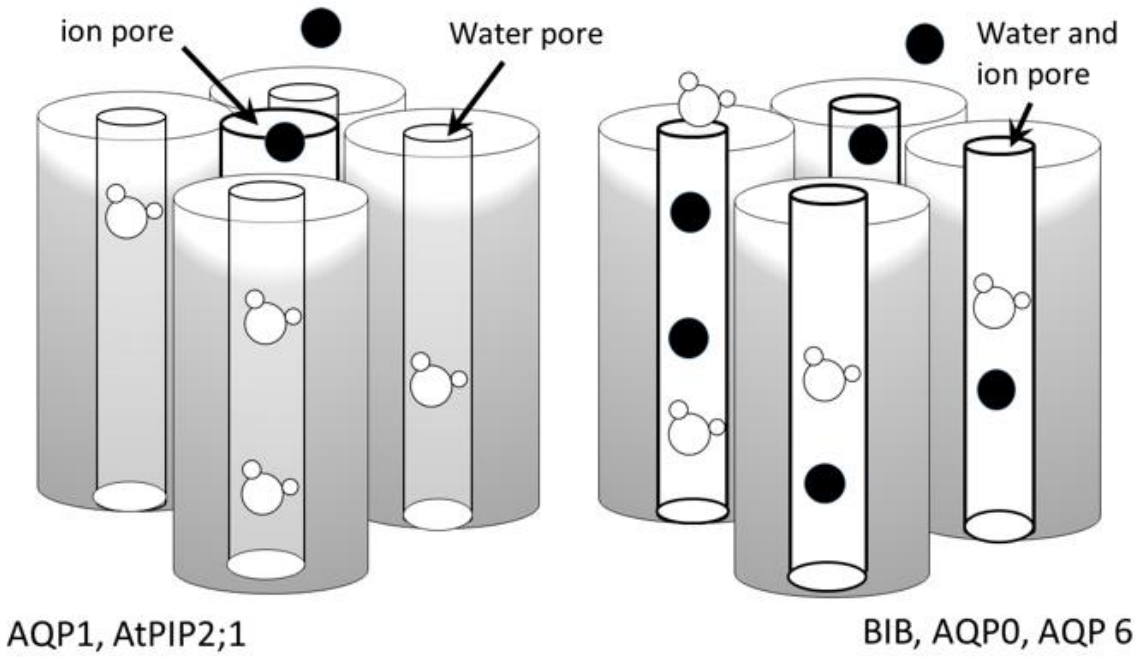


**Figure 3**

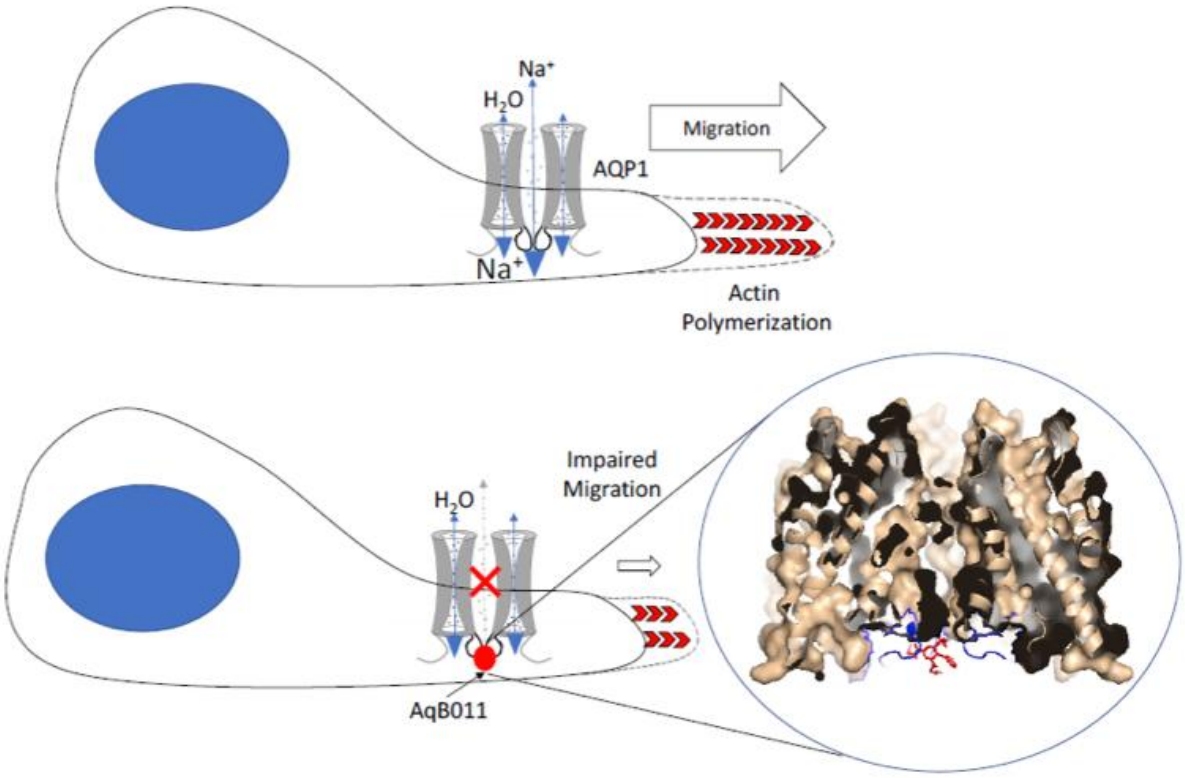

**Figure 4**

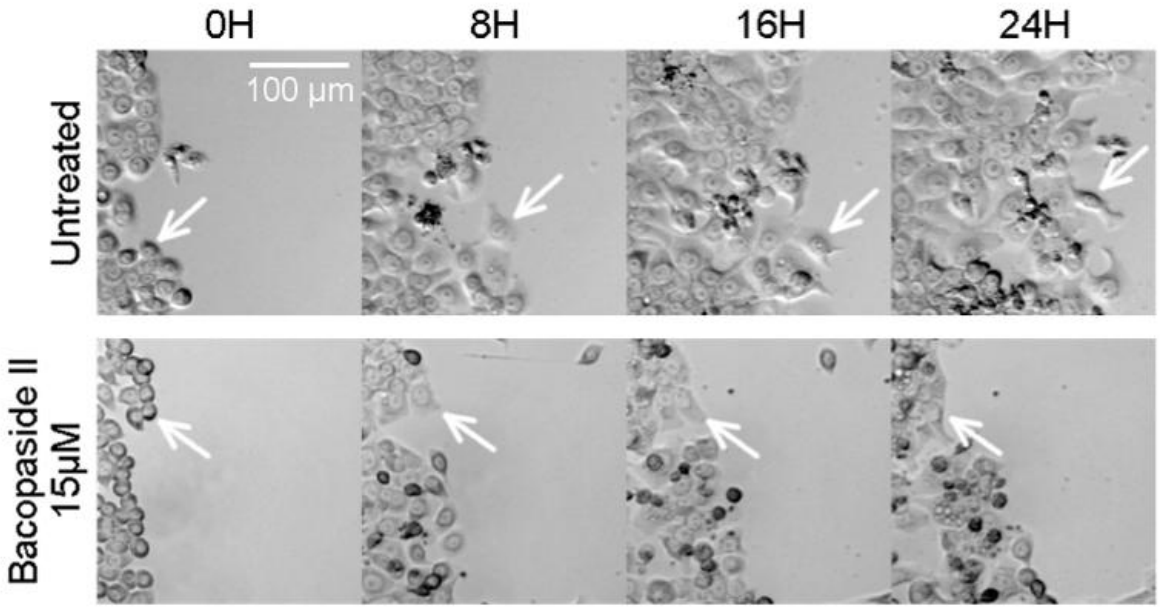


**Figure 5**

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BIB          GNLQAAISHSAALAAWERFGVEFILTFLVVLCYFVSTDPMKKFMGN-----SAASIGCAY
hAQP1        GNSLGRNDLADGVNSGQGLGIEIIGTLQLVLCVLATTDRRRRD----LGGSAPLAIGLSV
AtPIP2;7     TLGGGANTVADGYSKGTALGAEIIGTFVLVYTVFSATDPKRSARDSHIPVLAPLPIGFAV
AtPIP2;1     RYGGGANSLADGYSTGTGLAAEIIGTFVLVYTVFSATDPKRSARDSHVPVLAPLPIGFAV
AtPIP2;2     RYGGGANSLADGYNTGTGLAAEIIGTFVLVYTVFSATDPKRNARDSHVPVLAPLPIGFAV
                 .    : .      :. *:* *: :*   : :**  :          :   ** :
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