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Aquaporin Graphene Interface: Relevance to Point of Care Device for Renal Cell Carcinoma and Desalination

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Aquaporin Graphene Interface: Relevance to Point-of-Care Device for Renal Cell Carcinoma and Desalination

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Competing interests:

All authors declare no competing interests.

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Inspired by Suraj and Vikram Renugopalakrishnan

Abstract

Aquaporins superfamily of hydrophobic integral membrane proteins constitute water channels essential to the movement of water across the cell membrane maintaining homeostatic equilibrium. During the passage of water between the extracellular and intracellular sides of the cell, aquaporins act as ultra-sensitive filters. Due to their hydrophobic nature, aquaporins self-assemble in phospholipids and if a proper choice of lipids are made then the aquaporin biomimetic membrane can be used in the design of artificial kidney. In combination with graphene, aquaporin biomimetic membrane finds practical application in desalination and water recycling using mostly *E.coli* AqpZ. Recently, human aquaporin 1 has emerged as an important biomarker in renal cell carcinoma. At present the ultra-sensitive sensing of renal cell carcinoma is cumbersome and hence we are discussing usage of epitopes from monoclonal antibody as a probe for Point-of-Care device for sensing renal cell carcinoma by immobilizing the antibody on the surface of a single layer graphene as a microfluidic device for sensing renal cell carcinoma which is pursued in our laboratories.

Introduction

Permeation of water through the cell membrane is a critical event in maintaining an even hydrostatic pressure for billions of cells present in a living system. Cell membranes contain pores or "channels" which were not understood until Peter Agre discovered the first water channel for which he was awarded Nobel Prize in Chemistry, 2003 (https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2003/agre-lecture.html). For billions of cells to be able to function the coordination of water movement is required involving an intricate and elaborate communication between the cells. The signals transferred between cells consist of ions or small molecules. These trigger cascades of chemical reactions that cause our muscles to tense, our eyes to water – indeed, they control all our bodily functions.

(https://www.nobelprize.org/mediaplayer/index.php?id=550) (Movie 1).

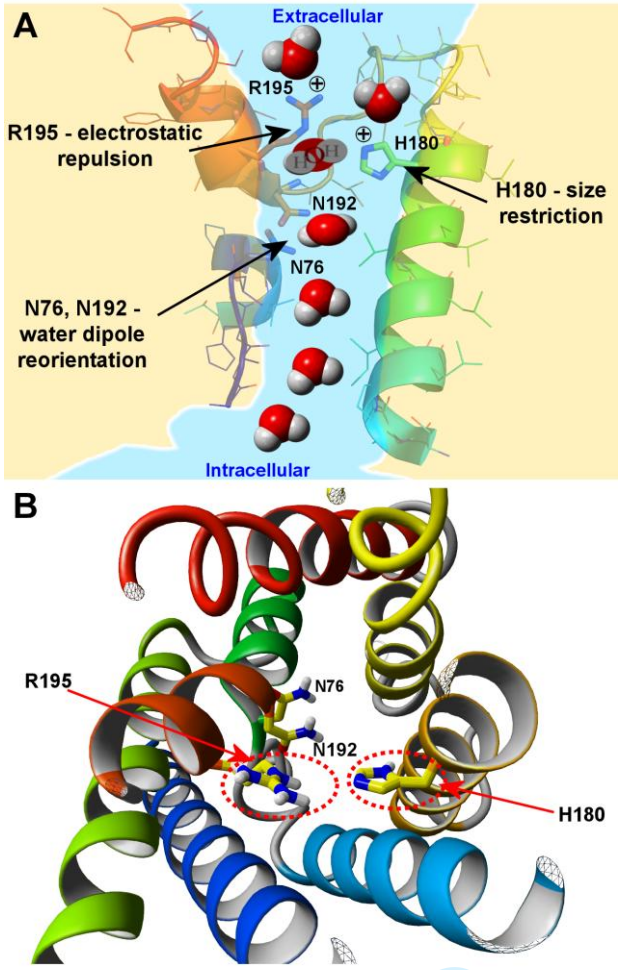

Fig. 1. Functional schematic view for water passage through human AQP1 (PDB id: 4CSK) [1]. **(A)** The extracellular vestibule and the intracellular vestibule of the channel contain water in bulk solution. Arginine-195 and histidine-180 provide fixed positive charges to repel proton passage. A single water molecule forms hydrogen bonds with the side chains of highly conserved asparagines-76 and -192. Partial positive charges are provided by the orientation of the two α helices that enter but do not entirely span the bilayer. **(B)** Top down view of aquaporin water channel. Residues facing the channel are colored in yellow. The residues H180 and R195 are labelled to show the size restriction gate.

Water channels consist of aquaporins, membrane proteins found in living cells, which facilitate highly efficient water transport in and out of the cells. Aquaporin water channels only allow water molecules to selectively pass through the channel and each aquaporin water channel transport up to one billion water molecules per second. The high water permeability characteristic of mammalian red blood cell membranes is now known to be caused by aquaporin, AQP1. This channel freely permits movement of water across the cell membrane, but it is not permeable to other small, uncharged molecules or charged solutes. AQP1 is a tetramer with each subunit similar to an hourglass containing an aqueous pore (Fig. 2A and 2B). Additionally, some aquaporins, e.g. human AQP5 (mainly localized in cells proximal to air-interacting surfaces) or spinach SoPIP2;1 incorporate gating mechanism. Channel closure can be a consequence of dephosphorylation of two conserved serine residues under drought, or of the protonation of a conserved histidine residue following a drop in cytoplasmic pH during flooding (Fig. 2C).

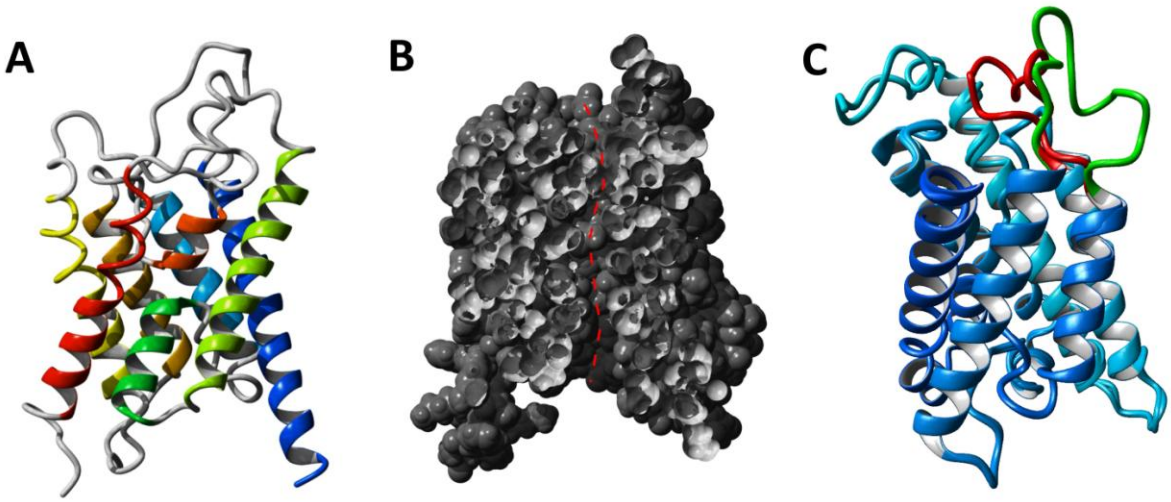

Fig. 2. Aquaporin crystal structure. **(A)** Hourglass shape of human aquaporin AQP1 (PDB id: 4CSK) [1]. The central part of the protein is composed of two

half-helices (green and orange). **(B)** Water pathway (red dashed line) across AQP1. The protein is showed as gray van der Waals spheres. **(C)** Open (loop in green) (PDB ID: 2B5F) and closed (loop in red) (PDB ID: 1Z98) [2] conformations of spinach aquaporin SoPIP2;1.

The aquaporins reported to date crystallizes as homo-tetramers with each monomer forming an independent transmembrane channel. Such tetramers display extended hydrophobic interactions between monomers [3]. However, the requirement for tetramerization of aquaporins that forms four apparently independent channels remains enigmatic [4]. The crystal structure of atomic resolution of human AQP1 tetramer [1] imposed into the lipid bilayer in the periodic simulation box is shown in Fig. 3.

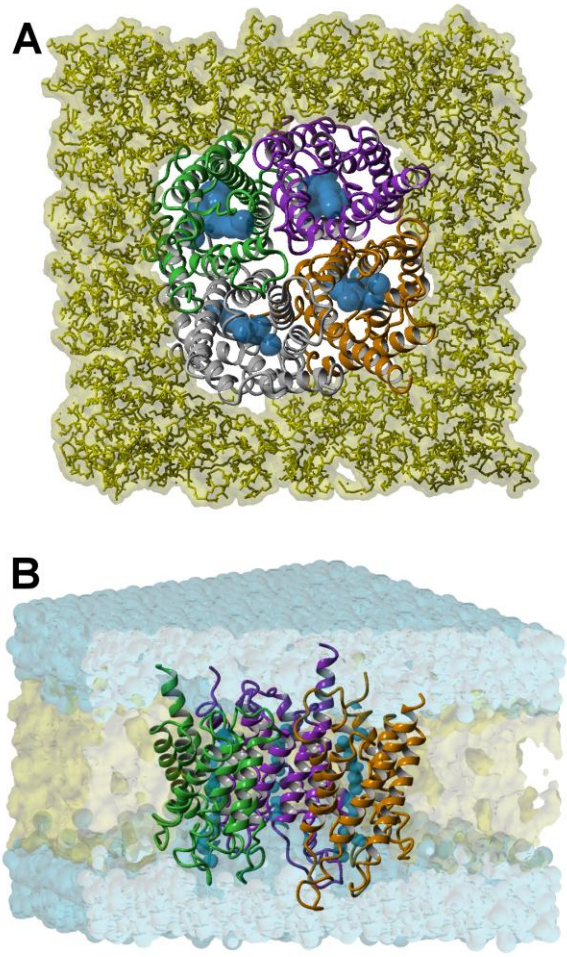

Fig. 3. Membrane protein simulation system. Top **(A)** and side view **(B)** of the tetramer simulation system of a human AQP1 (PDB id:4CSK) [1] embedded in a pure POPE bilayer. In the side view, the front monomer is removed for clarity. Water molecules in water channel of AQP1 are shown as transparent blue van der Waals spheres.

Aquaporin Superfamily - Major Intrinsic Proteins

Major intrinsic proteins comprise a large superfamily of transmembrane protein channels that are grouped together on the basis of homology. The MIP superfamily

includes three subfamilies: aquaporins, aquaglyceroporins and S-aquaporins. The aquaporins (AQPs) are water selective membrane channels expressed in almost every organism and involved in the bidirectional transfer of water and small solutes across cell membranes. The aquaglyceroporins are permeable to water, but also to other small uncharged molecules such as glycerol, urea or ammonia. The third subfamily include so called superaquaporins (S-aquaporins) or subcellular aquaporins, a third subfamily only present in animals but not in plants, fungi and bacteria with uncertain permeability [5].

Aquaporin are proteins with molecular masses around 30 kDa (monomer size) [6]. Three-dimensional structures of several aquaporins have been determined and the quaternary structures of the proteins reveal that they all form homotetramers where each monomer acts as a functional unit. Sequence homology similarity suggests functional unit of all members in this super family are predicted to have six hydrophobic, membrane spanning α-helices connected by five loops of variable length that delimit a polar channel with two wide vestibules and a narrow pore. Two of the connecting loops, namely B and E, interact with each other from opposite sides through two highly conserved (Asn-Pro-Ala) motif conserved throughout the aquaporin family forming a seventh transmembrane region that contributes to the pore region of aquaporins.

To date, 13 isoforms of aquaporins has been discovered in mammals (AQP0 – AQP12), 9 of which is localized in different parts of the renal tubular epithelium [7]. Two main groups of aquaporins are distinguished: (i) classical aquaporins, permeable only to water molecules (AQP0, AQP1, AQP2, AQP4, AQP5) and (ii) aquaglyceroporins, permeable for other small molecules, such as glycerol and ammonia (AQP3, AQP7, AQP9, AQP10) [8]. In addition, a third group has been recently isolated, the so called unorthodox aquaporins (AQP11 and AQP12), which

share low homology with other proteins from this family [9]. AQP6 and AQP8 are classified as unorthodox aquaporins; however, due to their ability to transport other small molecules, the present review will discuss them along with the rest of aquaglyceroporins located in the kidneys [7]. Aquaporin family are implicated in numerous physiological processes as well as the pathophysiology of a wide range of clinical disorder. The critical residues affecting channeling performance of AQP1 are collected in Table 1, while for other aquaporins in Table S1 in the supplementary material. Some mutations were also introduced artificially to get knowledge about mechanism of water permeability and/or to change selectivity.

Table 1. Mutations in AQP1 affecting its permeability and selectivity.

AQP1	C189S	C189S mutant of AQP1 induces the same CO_2 permeability as the wild-type AQP1 but that the C189S-dependent increase in CO_2 permeability is insensitive to p-chloromercuribenzene sulfonate (pCMBS).	[10,11]
AQP1	ΔT157, ΔT239 (lacking)	Inactivation of any of two PKC (protein kinase C) phosphorylation sites T157 and T239 abolishes the positive regulation of AQP1 water permeability by PKC.	[11,12]
AQP1 (human)	H180A/ R195V	This double mutant changes electrostatics of the selectivity filter region and facilitates the proton to entry from the bulk water into the narrow AQP1 channel. The double mutation drastically drops the overall free-energy barrier by roughly 20 kcal/mol via simultaneously relaxing the direct electrostatic interaction (by R195V) and dehydration effect (by H180A).	[13,14]
AQP1 (rat)	H180A	Replacing H180 with alanine increases the channel diameter and reduces the dehydration penalty for the proton. H180A mutant passed ammonia significantly faster than wild type. Mutation did not affect water permeability.	[15]
AQP1 (rat)	F56A/ H180A	Joint mutations of residues F56 and H180 in the selectivity filter of rat AQP1 have been shown to lower its selectivity. Replacement of both residues enlarged the maximal diameter of the aromatic/arginine (ar/R) constriction 3-fold and enabled glycerol and urea to pass. F56A/H180A mutant passed ammonia significantly faster than the wild type.	[15]
AQP1 (rat)	H180A/ R195V	Individual or joint mutations R195 in the selectivity filter of rat AQP1 have been shown to lower its selectivity experimentally. In particular the substitution of R195 was shown to allow the passage of protons. The double mutant did not affect water permeability but passed ammonia significantly faster.	[15]

AQP1 (rat)	R195S	The mutation changes the orientations of water molecules along the channel and therefore influence the proton permeability. Enlarged channel radius allows for the existence of two water molecules at the constriction region.	[15]
AQP1 (human)	Y186F, Y186A, Y186N	Mutants created to investigate whether a tyrosine residue in loop E of AQP1 was involved in water permeability. Y186F conferred high water permeability comparable to that seen in wt AQP1 while Y186A and Y186N mutants did not show significant canges.	[16]
AQP1 (human)	A73M	A73M-yeast expressing cells did not exhibit osmosensitivity in comparison with wt AQP1-expressing cells.	[17]

AQP1 Point-of-Care device

Aquaporins have important biological roles and have been implicated in several pathophysiological conditions suggesting a great translational potential in aquaporin-based diagnostics and therapeutics. Recently, overexpression of AQP1 has been associated with many types of carcinoma as a distinctive clinical prognostic factor. This has prompted researchers to evaluate the link between AQP1 and cancer biology [18]. AQP1 is overexpressed in multiple human cancers including that of biliary duct, bladder, brain, breast, cervix, colon, lung, nasopharynx and prostate. In case of colon cancer it has been suggested that AQP1 is especially involved in early stages of cancer tumorigenesis [19]. Additionally, its expression was reported to be associated with clinical characteristics known to be prognostic such as histological grade and status of lympho-vascular invasion and nodal involvement. These findings suggested a link between AQP1 and cancer biological functions, which act to drive cancer development and progression. Various hypotheses have been advanced to explain the critical role of AQP1 in tumorigenesis and especially resistance to apoptosis has been proposed as a part of the mechanism underlying enhanced cell proliferation of AQP1 expressing cells [20,21].

Testing for AQP1 alongside other urine biomarkers such as perilipin-2 (PLIN2), HIF-1α, carbonic anhydrase IX, and VEGF would allow for a more sensitive and specific diagnosis of renal cell carcinoma (RCC) as well as more accurate prognosis [22,23]. Urine concentrations of AQP1 or PLIN2 were not increased in patients with common non-cancerous kidney diseases, non-cancerous kidney tumors or bladder or prostate cancer. Thus, common kidney disease and non-renal urologic cancers do not confound the ability of AQP1 and PLIN2 to detect clear cell and papillary cancers, suggesting that these biomarkers have potential for population screening and/or differential diagnosis.

At the present time there exists no Point-of-Care (POC) device for an accurate and easy way of sensing AQP1 in urine. We have begun a design of AQP1, and other selected biomarkers detecting POC device based on a similar approach to sandwich ELISA (enzyme-linked immunosorbent assay - a plate-based assay technique) [24]. The complicated nature of standard Western blot procedures precludes its clinical implementation for renal cancer screening. While ELISA test is a solid-phase method and requires a solid support, usually a polystyrene microtiter plate, such plate would not be required in our method, since a single graphene sheet would play the role of solid support. A specific AQP1 antibodies [25] would be bound through a special linker molecules to the surface of a single layer graphene embedded in a microfluidic channel [26]. A scheme of single layer graphene attached to a peptide epitope of AQP1 and interacting with AQP1 is shown in Fig. 4. The urine sample would be sucked into those microfluidic channels and delivered to AQP1 specific antibodies immobilized on a graphene attached to field-effect transistor (FET) [26]. AQP1 molecules (if present in the urine sample) would bind to those antibodies changing graphene layer electrical potential. The changes of electrical potential will be measured and their magnitude will be

proportional to the number of AQP1 molecules bound to the graphene layer and will reflect the concentration of AQP1 in the urine sample.

The proposed method would not require the secondary antibody linked to an enzyme which is necessary in standard ELISA tests. The proposed method of AQP1 detection is more advantageous over ELISA and western blot because of its direct measurements. While it is expected to have similar accuracy as ELISA it much quicker and easier to perform (it does not involve long incubation times like ELISA and Western blots do). Additional detection of PLIN2 could increase assay efficiency and enable widespread implementation [27]. Further improvements in the AQP1 detection ELISA tests may potentially reduce the AQP1 background coming from common non-cancerous kidney diseases, bladder and prostate cancer, which can lead to false positive RCC tests.

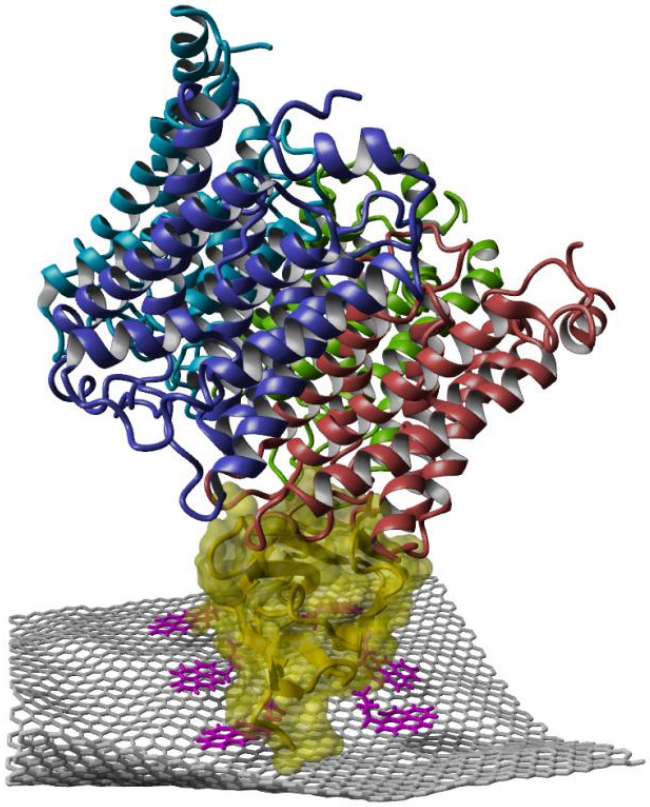

Fig. 4. Schematic draw of AQP1 antibody linked to graphene interacting with tetramer of AQP1. So far two antibodies were developed: linking to extracellular loop of AQP1 (198-GSAVLTRNFSN-208) and to intracellular loop (249-GQVEEYDLDDDINSRVEMKPK-269) [25].

The monitoring of RCC patients using a POC device measuring AQP1, and potentially other biomarkers levels could be also useful to track of disease progression since different levels of such biomarkers can be found at various stages of tumorogenesis. Recently, it was also found that serum levels of many biomarkers, including AQP1 and AQP4, was increased in response to injury in mice [28]. This suggest that new types of enhanced diagnostics in injury-related

neurological disorders, multi-system deficits, and possibly cancer could be developed.

Properties and applications of spinach aquaporin

Water permeability characteristics of spinach aquaporin (SoPIP2;1) have been well described [2,5], and therefore it is a good candidate for application in technology. The expression of aquaporins was up-regulated in response to drought and salinity, and conferred the water stress tolerance in plant. Aquaporins are involved in many functions of plants, including nutrient acquisition, carbon fixation, cell signaling and stress responses. The high selectivity and water permeability of SoPIP2;1 makes it particularly interesting for a biomimetic water desalination and water filtration technology.

Tertiary structure of SoPIP2;1 containing six tryptophan (Trp) residues in the primary sequence which provide an intrinsic fluorophores for analyzing structural fluctuations by fluorescence spectroscopy using Trp fluorescence emission spectrum which is sensitive to both the polarity and the dynamics of the environment surrounding the aromatic side chain. Plasencia *et al.* [5] have used CD spectroscopic studies to probe structural stability of spinach aquaporins, SoPIP2;1, and derived the conclusion that SoPIP2;1 can exist as a stable folded protein in nonionic OG detergent micelles solutions and that the protein can be transferred from detergent micelles solutions and reconstituted into selected phospholipid membranes preserving its structural characteristics. It is likely that more suitable reconstitution systems exist for SoPIP2;1 than those studied in the present work. In order to efficiently test a range of systems, new methods are called for. Presently, we are working on developing a new microscopic method

that will allow us to test at the same time the incorporation and distribution of the protein in different membrane systems and evaluate the yield of the incorporation and characterize the protein functionality.

Plant aquaporin channel gating is triggered by dephosphorylation of two conserved serine residues, or by the protonation of a conserved histidine residue. X-ray structure of the spinach plasma membrane aquaporin SoPIP2;1 in its closed conformation at 2.1 Å resolution and in its open conformation at 3.9 Å resolution, has been validated by molecular dynamics simulations [2]. In the closed conformation loop D caps the channel from the cytoplasm and thereby occludes the pore. In the open conformation loop D is displaced up to 16 Å and this movement opens a hydrophobic gate blocking the channel entrance from the cytoplasm (Fig. 5).

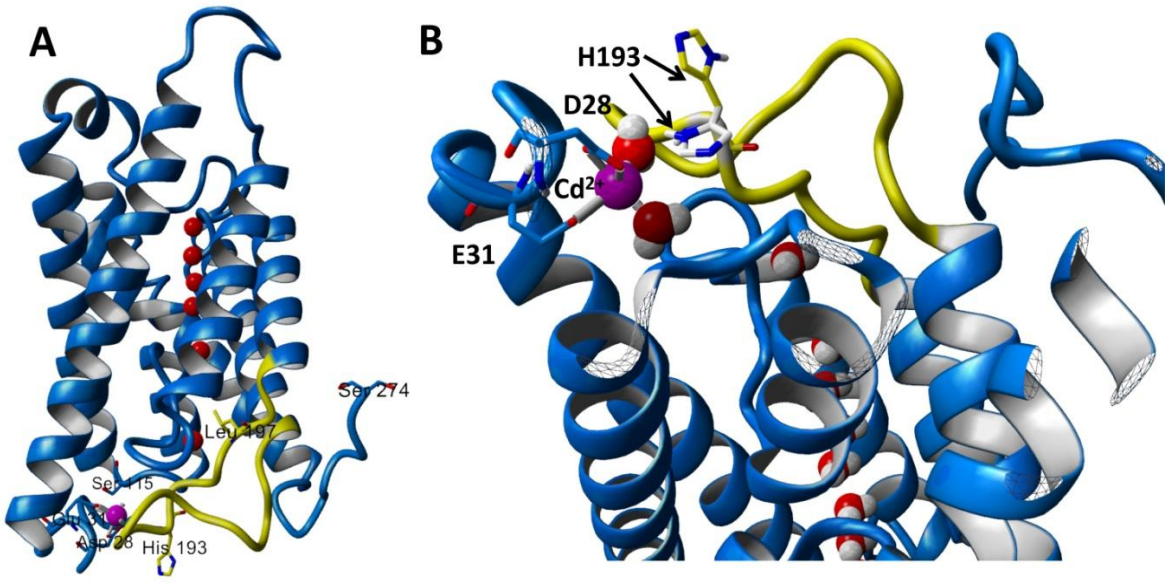

Fig. 5. Proposed gating mechanism for AQP (spinach aquaporin SoPIP2;1) (PDB id: 1Z98) [2]. **(A)** Overview of AQP in the closed conformation at pH 8. Interactions between Loop D (in yellow) and a Cd^{2+}-binding site at the N-terminus inserts a hydrophobic plug indicated by L197, thereby occluding the water

conducting pore. The Cd^{2+}-ion and water molecules in the water conducting channel are shown as yellow and red spheres respectively. Residues involved in gating by phosphorylation (S115 and S274) as well as gating by pH (H193) are indicated. **(B)** Close-up view of H193. In the protonated state, an alternative rotamer of the H193 side-chain (in white) may be adopted which is within hydrogen bonding distance of D28.

Scaling up expression and purification of recombinant aquaporins

AQP mediated water transport is a prominent example of how Nature has evolved an effective mechanism for purifying water, and many technologies based on biomimetic membrane transport is now attracting considerable commercial interest. However, successful reconstitution and stabilization of functional proteins in biomimetic membranes depends on suitable choices of both detergent and host lipid membrane components.

Recombinant aquaporins have been expressed only in lab-scale quantities for screening, functional, regulatory or structural studies [29,30]. One of the main obstacles in protein production is that membrane protein overexpression *in vivo* is hampered by their complex structure, hydrophobic transmembrane regions, host toxicity, and the time consuming and low efficiency refolding steps required. Recent developments of high-expression systems may however provide insights into how large-scale AQP production may be realized. These include E. coli, Saccharomyces cerevisiae, Pichia pastoris, and baculovirus/insect cell based systems, for a recent review see [31].

Status of aquaporin membrane development

Kumar *et al.* [32] suggested that membranes with very high permeability and salt rejection may be constructed based on aquaporin protein function. Based on the measured water permeability of AqpZ containing proteoliposomes, these authors postulated that AqpZ based biomimetic membranes can potentially achieve a membrane permeability as high two orders of magnitude more permeable compared to existing commercially available seawater reversed osmosis (RO) membranes. However a major issue still remained unresolved since the membrane is constructed from nanoscale elements (the aquaporins) how is the biomimetic membrane scaled-up and stabilized to 2 dimensions suitable for industrial applications. Several design strategies have recently been proposed, see Fig. 6.

These include membranes established across multiple micron scale apertures either as free-standing lipid or polymer membranes or as membranes stabilized by polymeric support materials. Other approaches rely on nanoporous support material onto which membranes are deposited. These include charged lipid vesicle depositions onto commercially available nan filtration membranes where the recipient surface was either cross-linked polyamide or sulfonated polysulfone both negatively charged at pH 7; rupture of aquaporin containing polymersomes on methacrylate functionalized cellulose acetate membranes detergent-stabilized His-tagged aquaporin added to monolayers with nickel-chelating lipids and proteopolymersome deposition onto polycarbonate track-etched substrates coated with gold and functionalized with photo-active acrylate groups.

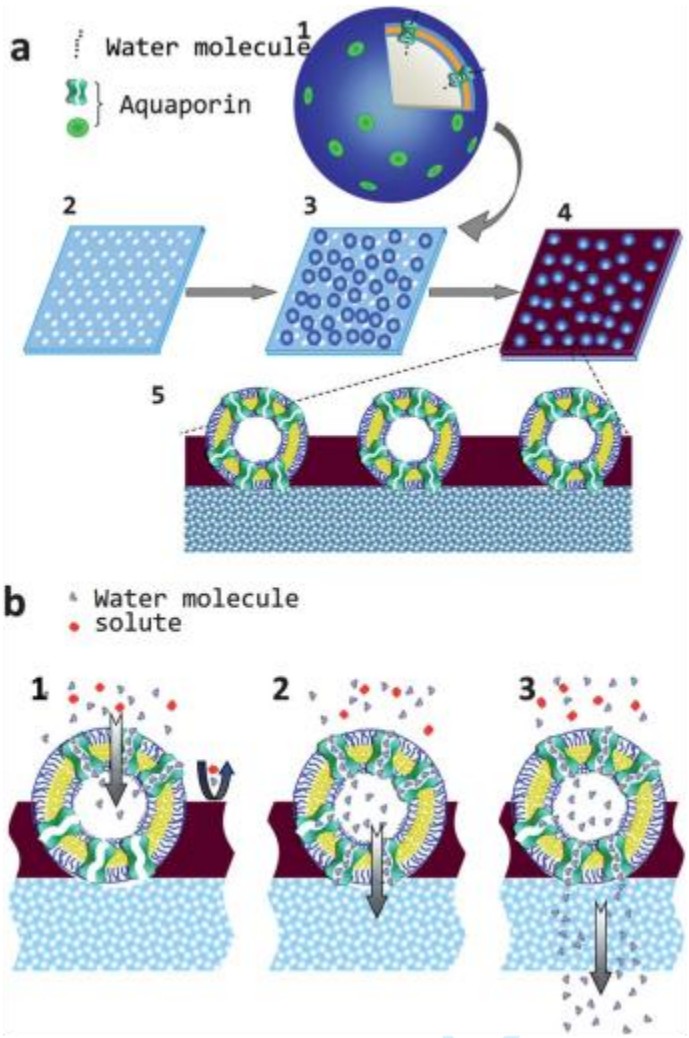

Fig. 6. Schemes for the fabrication and water purification mechanism of *E.coli* AqpZ-vesicle-imprinted membrane. (a) Schematic diagram of the AqpZ-vesicle-imprinted membrane preparation: (1) AqpZ-polymer vesicles, (2) porous cellulose acetate membrane substrate, (3) AqpZ vesicles immobilized on the porous membrane, (4) AqpZ-vesicle-imprinted membrane, and (5) cross-section of the AqpZ-vesicle-imprinted membrane. (b) Water purification mechanism of the AqpZ-vesicle-imprinted membrane under pressure. Water molecules in the feed solution will penetrate the entire membrane in 3 steps: (1) passing from the feed solution to the vesicles through the AqpZ water channel located at the polymer bilayer facing the feed solution, (2) passing from the vesicles to the substrate

membrane through the AqpZ located at the polymer bilayer facing the substrate membrane, and (3) penetrating the porous hydrophilic substrate membrane into the permeate solution [33]. Copyright Permission.

Forward Osmosis membrane

Forward Osmosis (FO) is a membrane separation technology powered by osmotic pressure gradient. Unlike reverse osmosis (RO) that needs external pressure to function, forward osmosis (FO) is driven by osmotic pressure difference across a semipermeable membrane. It has recently gained wider attention in many applications, such as seawater desalination and power generation [29,30,34-37]. There are several FO plants operating now for seawater desalination using full scale FO membranes with satisfactory results reported [38]. FO processes operate close to the atmospheric pressure, and relies on osmotic pressure gradient across a semi permeable membrane for fresh water extraction from feed saline water. Fresh water crosses the FO membrane from the feed to the draw solution side of the membrane and diluting the draw solution. Diluted draw solution is, typically, sent to thermal or membrane treatment processes for fresh water extraction and draw solution regeneration and reuse. An ideal draw solute should be characterized by the ability to ensure high osmotic gradient, substantial water flux, and efficient recovery at minimal energy consumption [39]. For osmosis-driven desalination, an ideal draw solute should have zero toxicity and low cost among other its characteristics [40]. Many solutes suitable for water desalination had been developed, among which some are classified as 1) inorganic-based draw solutions (solutes: $CaCl_2$, $KHCO_3$, $MgCl_2$ etc.), 2) organic-based draw solutions - the solutes are non-electrolytes but can generate high osmotic pressure due to their high

solubility (example: glucose, fructose, ethanol). In recent years, highly hydrophilic nano-sized magnetic particles, functionalized by polyacrylic acid have been discovered to be crucial in the application of draw solutes in FO desalination as this engineering can yield high osmotic pressure and high water flux [41]. Magnetic separators can be used to recycle the magnetic particles. In general, FO process does not require high energy for operation and most of the energy consumption would be incurred in the regeneration process [42,43]. It can be coupled with membrane and thermal processes for seawater treatment, for example FO has been suggested for the pretreatment of feed water to thermal and membrane processes [44,45] to provide high feed quality especially in case of high fouling feed waters. FO process can operate at high efficiency without need for frequent cleaning due to the reversible fouling nature. However, there are several challenges facing the application of FO process; one of these challenges is the FO membrane; initially, membrane used in the FO process were inefficient because of the severe Concentration Polarization (CP) phenomenon which takes place on the feed and draw solution side of the membrane and resulting in a sharp decrease in water flux across the FO membrane.

There was plethora of attempts to develop an efficient FO membrane [45-48]; most of these attempts were laboratory scale experiments. Results shown in Table S2 in supplementary materials reveal that experimental work was successful in the development of high permeability FO membrane which achieved a staggering water flux of 32 L/m^2h using 0.5 mol NaCl draw solution and DI water as the feed solution. This was almost double water flux achieved by CTA HTI membrane using same salinity gradient resource (Table S2 in supplementary materials). Using modified PVDF membrane enhanced water flux because of the larger pore size but

caused higher reverse salt diffusion. One of strategies to improve the performance of the FO process is by increasing the membrane permeability that compensates flux loss due to internal and external concentration polarization. Aquaporin membranes exhibit water flux higher than that in the traditional CTA and polyamide membranes. Using such high water permeability membrane will revolutionize the FO process. The low fouling tendency and operating pressure of the FO process will increase the life time of aquaporin membrane and improve the performance of FO process. Future research work should focus on the development of an aquaporin membrane with properties suitable for the FO process such as high water flux and thin porous structure to reduce the effect of concentration polarization.

Aquaporin and graphene RO membranes

Nanometer-scale pores in single-layer of graphene has been shown to be an effective sieve in separating NaCl from water (Fig. 7).

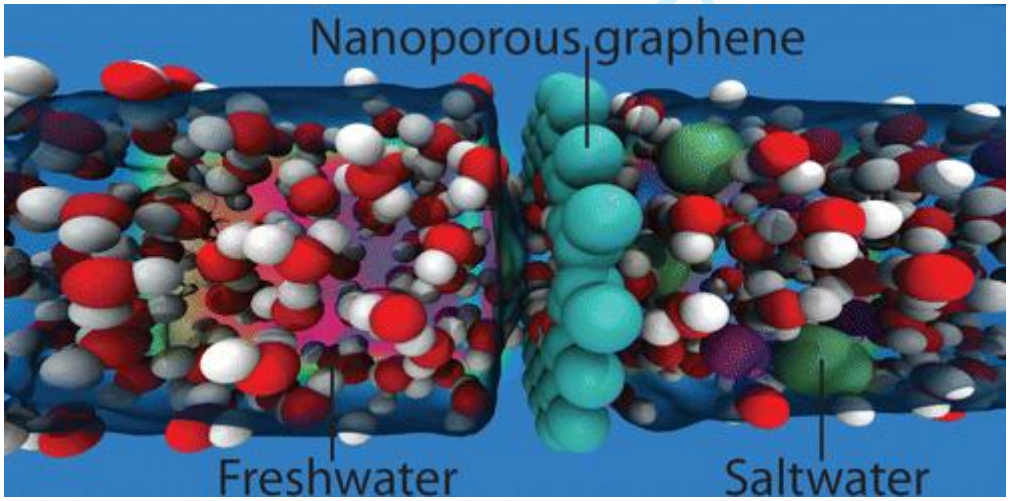

Fig. 7. Scheme of separating NaCl from water using nanoporous graphene [49]. Copyright Permission.

Cohen-Tanugi and Grossman [49] have reported from classical molecular dynamics the desalination performance of membranes as a function of the membrane's ability to prevent the salt passage depends critically on pore diameter (pores size varies from 1.5 to 62 $Å^2$) with adequately sized pores allowing for water flow while blocking ions. According to simulations results the maximum pore diameter allowing to reject salt ions is 5.5 Å (pore size ~24 $Å^2$), while the minimum pore diameter required for water passage is 3.8 Å (pore size 11.3 $Å^2$) [6]. The simulations results also indicate that the water permeability of nanoporous graphene is several orders of magnitude higher than conventional reverse osmosis membranes, and that this material may have a valuable role to play for water purification.

The lipid bilayer with aquaporin channels imprinted in it should have somewhat smaller permeability than nanoporous graphene, because of the smaller pore size and similar or smaller pore density (depending on how many aquaporin units are immersed in the membrane). But still we expect a permeability improvement, relative to a standard RO membrane, of one to two orders of magnitude. Under these conditions the energy required for purifying water will drop significantly, potentially making desalination a much more widely applicable technology. Although it is likely that fluxes will still be modest, low operating pressures could make the technology commercially attractive due to low energy cost. The challenges for aquaporin membranes will be robustness and longevity.

Conclusions

Our interest in AQP1 stems from its important role in renal cell carcinoma and a critical need to design efficient POC devices. Due to application of AQP1 specific antibodies immobilized on a graphene attached to field-effect transistor, the measurement process would be much simpler and faster than in standard biomedical assays. Recombinant aquaporin (from spinach or bacteria *E.coli*) films can potentially improve water desalination at reduced operational costs and low energy consumption. Despite the high performance of aquaporin membrane, there is still long time before these membranes can be available for commercial application. Techno-economical issues need to be addressed before aquaporin technology can be applied for large desalination projects.

Authors' Contributions

JJ performed all computations required for molecular structures, made all figures of proteins and graphene and participated in writing of paper; AS selected literature information and completed Table 1. SF analyzed computed systems and participated in writing of paper. AA and AOS contributed the write up on FO and RO in desalination. JRE contributed in the development of ideas on renal cell carcinoma detection. PL in the expression of aquaporin and site directed mutations. KR and BR on the development of cartridges using aquaporin biomimetic membrane, SR contributed idea on fundamental mechanisms in aquaporin-graphene interface. PMA for the development of single layer graphene. Initial ideas on aquaporins is credited to VR. Expression of aquaporins from plasmids in *Saccharomyces Cerevisiae* and large number of thermostable aquaporins was performed in VR laboratories. VR coordinated the study and drafted the manuscript. All authors gave final approval for the publication.

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