

MEMBRANE COMPRISING CONSTITUTIVELY OPEN AQUAPORINS

Technical Field

5 The present invention relates to a constitutively open aquaporin. Further, it relates to a membrane comprising such an aquaporin and a use of such membrane to purify water. The present invention also relates to a method of converting aquaporins, to constitutively open aquaporins.

Background

10 Naturally occurring biological membranes have only limited intrinsic water permeability cells maintain the flux of water into and out of the cell via a family of water-specific, membrane protein channels called aquaporins.

Members of the aquaporin family are well known in literature, and found in archaea, eubacteria and eukaryotes, including fungi, animals and plants. They serve an astonishing variety of physiological functions and are easily identified by sequence similarity across all kingdoms of life. In higher eukaryotes, water transport activity of aquaporins is frequently regulated by phosphorylation, pH and osmolarity. Aquaporins in plants and animals are highly conserved and form large protein families with 35 members in higher plants and 13 members in humans.

20 Based upon phylogenetic analyses, plant aquaporins are further divided into four subfamilies and their presence in primitive plants such as the bryophyte *Physcomitrella patens* implies that this specialization was already present in an ancient plant-ancestor.

There are 13 remarkably conserved plasma membrane aquaporins (Plasma membrane Intrinsic Proteins or PIPs) which are all regulated, and these further separate into two distinct phylogenetic groups (PIP1 and PIP2).

Synthetic membranes with aquaporins incorporated in their structure have been described in literature, e.g. in US 7,857,978 B2, US 2009/0007555 A1, and EP 1 885 477 B1, as well as use of such membranes for purifying water.

30 However, the synthetic membranes are often not very effective, since the natural limitations of aquaporins are also present in the synthetic membranes comprising such aquaporins.

Hence, an improved membrane, device or use for purifying water would be advantageous.

Summary

Accordingly, the present invention preferably seeks to mitigate, alleviate or eliminate one or more of the above-identified deficiencies in the art and disadvantages singly or in any combination and solves at least the above mentioned problems by providing a device, a method and a use, according to the appended patent claims.

The general solution according to the invention is to utilize constitutively open aquaporins in a synthetic membrane.

According to a first aspect of the invention a membrane comprising a constitutively open His-tagged aquaporin is provided.

The constitutively open His-tagged aquaporin may have at least 90 %, such as at least 95, 97 or 99%, sequence homology with SEQ ID NO: 2.

The membrane may comprise a constitutively open His-tagged aquaporin having amino acid sequence according to SEQ ID NO: 2.

In an embodiment, the aquaporin of the membrane originates from *Spinacia oleracea*, *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* or *Physcomitrella patens*.

The aquaporin may comprise a sequence having at least 90 %, such as at least 95, 97 or 99%, sequence homology with any of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, or SEQ ID NO: 30.

The aquaporin may comprise a C-terminal His-tag having at least 90 %, such as at least 95, 97 or 99%, sequence homology with SEQ ID NO: 3.

In an embodiment, the aquaporin comprises the sequence according to SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, or SEQ ID NO: 30, and a C-terminal His-tag having according to SEQ ID NO: 3.

According to a second aspect of the invention, a device for purifying water is provided. The device comprises an inlet connected to a first chamber, for receiving dirty water, and an outlet connected to a second chamber for dispensing clean water, and a

water purifying membrane comprising the membrane according to according to the first aspect, arranged in between the first and the second chamber.

According to a third aspect of the invention, use of the membrane according to the first aspect or a device according to the second aspect, for purifying water is
5 provided.

According to a fourth aspect of the invention, a method for producing a membrane according to the first aspect is provided, said method comprising the steps of: producing a His-tagged aquaporin, which is constitutively open; and incorporating the aquaporin into a membrane.

10 The present invention has the advantage over the prior art that it offers increased water permeability without negatively affecting the water purification.

Brief Description of the Drawings

These and other aspects, features and advantages of which the invention is
15 capable of will be apparent and elucidated from the following description of embodiments of the present invention, reference being made to the accompanying drawings, in which

Fig. 1 is a graph according to an embodiment of the invention.

Description of embodiments

Several embodiments of the present invention will be described in more detail below with reference to the accompanying drawings in order for those skilled in the art to be able to carry out the invention. The invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth
25 herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. The embodiments do not limit the invention, but the invention is only limited by the appended patent claims. Furthermore, the terminology used in the detailed description of the particular embodiments illustrated in the accompanying drawings is not intended
30 to be limiting of the invention.

The following description focuses on an embodiment of the present invention applicable to a membrane comprising a constitutively open His-tagged aquaporin and in particular to a membrane comprising a constitutively open His-tagged aquaporin derived from *Spinacia oleracea*, SoPIP2;1 (SEQ ID NO: 1). However, it will be
35 appreciated that the invention is not limited to this application but may be applied to

many other His-tagged aquaporins including for example aquaporins derived from, or originating from, *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* or *Physcomitrella patens*.

Closure of the plant aquaporin SoPIP2;1 (SEQ ID NO: 1) of spinach (*Spinacia oleracea*), formerly called PM28A, has been reported to be triggered by the dephosphorylation of two serine residues: Ser115 in the cytosolic loop B (conserved as Ser in 12, and as Thr in 1, of the 13 *Arabidopsis* PIPs) and Ser274 in the C-terminal region (conserved as Ser in seven, and as Thr in one, of the eight *Arabidopsis* PIP2s). Both residues are situated in consensus phosphorylation sites.

The so far overexpressed and purified aquaporins of plant and human origin have been shown to be functional when reconstituted into artificial membranes and have been stable for several months and no proteolytic degradation have occurred during this time. Aquaporins (e.g. SoPIP2;1 (SEQ ID NO: 1)) reconstituted into artificial membranes in the form of proteoliposomes are stable for month.

To have a continuous access to large amounts of pure and functional aquaporin is crucial for the development of water permeable biomimetic membranes. Purification from natural sources is limited by low expression levels and difficulties to separate closely related isoforms of aquaporins.

Heterologous overexpression in the yeast *Pichia pastoris* has proven to be an efficient method to circumvent these problems and the expression of the spinach aquaporin SoPIP2;1 (SEQ ID NO: 1) was the first demonstration of the potential of this system for membrane proteins (Karlsson et al. FEBS Lett 537, 68-72, 2003). The aquaporin was cloned into the expression vector allowing the addition of a stretch of histidines at the C-terminus of the protein, facilitating the purification of the overexpressed aquaporin using a Ni-affinity column. This resulted in the first published report of a heterologously overexpressed, purified, crystallized and structurally characterized eukaryotic membrane protein (Kukulski et al. J Mol Biol 350. 611-616, 2005). The high yields of a characterized highly permeable aquaporin of plant origin elected this protein as the best candidate for the development of the biomimetic membranes.

SoPIP2;1 (SEQ ID NO: 1) belong to one of the best characterized classes of aquaporins, the PIPs. In contrast to most aquaporins where the regulation of the permeability remains to be explored, PIPs are known to be gated by phosphorylation of serine amino acids causing the channel to open and by protonation of a histidine amino

acid or by binding of Ca^{2+} causing the channel to close (Törnroth-Horsefield et al. Nature 439, 688-694, 2006).

Bioinformatics searches identified a naturally existing isoform, AtTIP2;1, from the plant *Arabidopsis thaliana* as potential alternative to SoPIP2;1. The cDNA encoding AtTIP2;1 was successfully cloned and the protein was overexpressed in *Pichia pastoris* with a N-terminal His-tag followed by a TEV protease site to allow removal of the affinity tag if necessary. After optimization of the solubilization and purification the yield of AtTIP2;1 corresponded to about one third of SoPIP2;1 (SEQ ID NO: 1).

It was confirmed that the purified AtTIP2;1 is water permeable when reconstituted in proteoliposomes, but quantitative comparison of the water transport indicated that the permeability of AtTIP2;1 is approximately two thirds of the permeability of SoPIP2;1 (SEQ ID NO: 1). In contrast the thermal stability of AtTIP2;1 is much higher, with a melting point of 86° C compared to 57° C for So-PIP2;1.

SoPIP2;1 (SEQ ID NO: 1) also named PM28A was the first aquaporin shown to be gated by phosphorylations (Johansson et al. Plant Cell 10, 451-459, 1998). Later on it was shown that PIPs are also gated by pH and Ca^{2+} (Tournaire-Roux et al. Nature. 425, 393-397, 2003; Alleva et al. J Exp Bot, 57, 609-621, 2006) and recently this was also demonstrated on purified AtPIP2;1 reconstituted in proteoliposomes (Verdoucq et al. Biochem J, 415, 409-416, 2008). Since all of the amino acid residues that are important for the pH and Ca^{2+} gating of PIPs are conserved also in SoPIP2;1 it is reasonable to expect a similar regulation. Surprisingly this was not found, instead the permeability of the His-tagged SoPIP2;1 (SEQ ID NO: 2) in proteoliposomes was unaffected both by changes in pH and Ca^{2+} (Fig. 1). As can be seen in Fig. 1, the samples at 1mM Ca^{2+} , pH 7.5, pH 5.86, pH 7.01 and pH 6.07 lead to substantially the same curve, which is higher than the control curve in all time points except 0. It seems most likely that the C-terminal His-tag is converting SoPIP2;1 into a stable open conformation, i.e. constitutively open, since the gated AtPIP2;1 and the protein used to generate closed structure of SoPIP2;1 was lacking any His-tag whereas the open structure of SoPIP2;1 was generated from SoPIP2;1 with a C-terminal His-tag.

Overexpression and purification of aquaporin

The SoPIP2;1 aquaporin was overexpressed and purified as described by Karlsson et al. FEBS Lett 537, 68-72, 2003.

A *P. pastoris* expression kit, including vectors and host cells, was purchased from Invitrogen. The *pm28a* cDNA (GenBank accession number L77969) was

amplified with the forward primer *EcoRI*-YPM28A and the reverse primer *XbaI*-PM28A. The PCR product was cloned into the *P. pastoris* vector pPICZB and the resulting plasmid pHT-PM28A-PICZ was sequenced. The 22 amino acids added to the C-terminus of HT-PM28A are LEQKLISEEDLNSAVDHHHHHH and contain beside
5 the 6xHis tag a myc antibody epitope. A second construct was made using the forward primer *EcoRI*-YPM28A and the reverse primer PM28A-REV. The reverse primer has the original stop codon after PM28A and a *Bgl*II restriction site. The PCR product was cloned into pPICZB and the resulting plasmid pPM28A-PICZ was sequenced.

PM28A constructs were transformed into the wild-type *P. pastoris* strain X-33
10 and transformants with the highest expression according to immunostaining (TetraHis antibodies, Qiagen) were selected and grown on a large scale. Briefly, 200 ml of buffered glycerol complex medium was inoculated, and the culture was incubated at 30°C with shaking at 225 rpm overnight. The cells were pelleted by centrifugation and resuspended in 1200 ml of buffered methanol complex medium to obtain an OD600
15 =1.0. Cells were grown in baffled flasks at 30°C with shaking at 225 rpm. Additional (0.5% v/v) methanol was added to the culture every 24 h to maintain induction. The cells were harvested by centrifugation after 72-120 h and stored at -80°C.

Cell pellets were thawed and resuspended in breaking buffer (50 mM sodium phosphate buffer, pH 7.5, 5% glycerol). The cells were disrupted by three passages
20 through a French pressure cell at 16 000 psi and lysates were clarified by centrifugation at 3000 rpm for 30 min. Membranes were collected by centrifugation at 200 000xg for 120 min at 4°C, resuspended in breaking buffer and stored at -80°C. Between 400 and 500 mg of crude membrane protein per liter of cell culture, or about 13 mg/g of cells, was routinely obtained. Peripheral membrane proteins and proteins adhering to the
25 membranes were removed by urea/alkali treatment (such as described in Fotiadis, D., Jenö, P., Mini, T., Wirtz, S., Müller, S.A., Frayse, L., Kjellbom, P. and Engel, A. (2001) J. Biol. Chem. 276, 1707- 1714). The stripped membranes were resuspended in 20 mM HEPES-NaOH, pH 7.8, 50 mM NaCl, 10% glycerol, 2 mM L-mercaptoethanol (buffer A) at a protein concentration of about 10 mg/ml and stored frozen at -80°C until
30 further use.

Stripped membranes containing 20 mg of protein were diluted to a protein concentration of 2 mg/ml and solubilized in 3% octyl- β -D-thioglucopyranoside (OTG) by dropwise addition from a stock solution of 10% OTG in buffer A. After 30 min at room temperature, unsolubilized material was pelleted at 160 000xg for 30 min.

35 Solubilized protein was mixed for 2 h at 4°C with 2 ml of Ni-NTA agarose slurry

(Qiagen) preequilibrated with 20 mM HEPES-NaOH, pH 7.8, 300 mM NaCl, 10% glycerol, 2 mM β -mercaptoethanol, 0.4% OTG (buffer B) containing 10 mM imidazole. The non-bound protein fraction was removed by centrifugation and the Ni-NTA agarose was washed with buffer B+30 mM imidazole. The proteins were eluted with 1 ml of
5 buffer B+300 mM imidazole by mixing for 1 h at 4°C. Four additional elution steps were performed.

As will be appreciated by a person skilled in the art, the above method may be used to purify any of the aquaporins disclosed herewith.

The detergent used for solubilisation of SoPIP2;1 (SEQ ID NO: 1) may be
10 changed from OTG to OG, to avoid potential problems associated with precipitation of the detergent at low temperatures. More importantly, the Hepes buffer with imidazole may be exchanged for PBS at pH 7.5 to allow CD measurements and biotinylation of the protein and 10% of glycerol was added and recommended in all samples that were going to be frozen. The overall loss of SoPIP2;1 (SEQ ID NO: 1) in the extra steps
15 added to the purification scheme was estimated to around 25%. This have not constituted a major problem since large amounts of starting material have been available but the extra step and the loss obviously resulted in an increase of the work required to deliver the same amount of protein.

20 **Incorporation of aquaporins into membranes**

The possibilities to incorporate recombinant aquaporin molecules in different types of industrial membranes for water filtration are being explored. In this, the nature may be used as a model for the development of a novel nanobiotechnological water membrane technology. Pilot membrane systems may be developed for three
25 applications: A water purification application, an ocean energy application (salinity gradient energy) and an application for industrial wastewater reclamation and reuse.

As will be appreciated by a person skilled in the art, any kind of membrane suitable for purifying water, such as comprising a biological membrane, lipid membrane etc. with incorporated aquaporins, such as disclosed in US 7,857,978 B2, US
30 2009/0007555 A1, EP 1 885 477 B1, or Helix Nielsen, Anal Bioanal Chem (2009) 395:697–718, may be used to incorporate a constitutively open His-tagged aquaporin according to embodiments of the invention.

Thus, according to an embodiment, a method for producing a membrane according to embodiments of the invention is provided, said method comprising the

steps of: producing a His-tagged aquaporin, which is constitutively open; and incorporating the aquaporin into a membrane.

A high stability of the aquaporin in the biomimetic membrane is desirable since a denaturation of the protein would compromise the permeability of the membrane. The thermal stability of SoPIP2;1 have been investigated using Circular Dichroism spectroscopy and SDS-PAGE to follow the denaturation and aggregation. As mentioned above, the melting temperature of solubilized SoPIP2;1 was lower than the extremely stable AtTIP2;1. However, in proteoliposome membranes the stability of SoPIP2;1 is increased to above 70°C. Preliminary data also indicate that the thermal stability is higher at high pH relative to low pH.

Aquaporin proteins are functioning as water-specific channels in cell membranes. Genes encoding aquaporin proteins have been inserted in a specific yeast strain and the aquaporin protein produced and purified in its functional form and used for constructing the water-specific membranes. The gene coding for the spinach plasma membrane aquaporin SoPIP2;1 (SEQ ID NO: 1) has been cloned into the yeast *Pichia pastoris*, overexpressed and purified. Without buffer exchange the yield is about 20-30 mg pure SoPIP2;1 per preparation.

SoPIP2;1 with a C-terminal His-tag (SEQ ID NO: 2) has recently been shown to be constitutively open.

Thus, according to an embodiment a constitutively open His-tagged aquaporin having at least 90 %, such as at least 95, 97 or 99%, sequence homology with SEQ ID NO: 2 is provided.

Another embodiment relates to a constitutively open aquaporin having amino acid sequence according to SEQ ID NO: 2.

As described above, the plant aquaporin AtTIP2;1 have been overexpressed and purified in high yields. AtTIP2;1 was shown to be an active water channel when reconstituted in proteo-liposomes. AtTIP2;1 is most likely a constitutively open isoform according to measurements in proteoliposomes.

Aquaporins from *Zea mays*, *Oryza sativa* or *Physcomitrella patens* may also be useful for the purpose of the invention.

As discussed above, there are 13 remarkably conserved plasma membrane aquaporins (Plasma membrane Intrinsic Proteins or PIPs) which are all regulated. These may further be separated into two distinct phylogenetic groups (PIP1 and PIP2).

According to a non-limiting theory of the inventors, the constitutively open conformation of aquaporin is achieved when the C-terminal His-tag prevents interaction

between Ser274 of aquaporin, and the next monomer which can be seen in a closed conformation, depending on the exact type of aquaporin. Ser274 is however conserved in all aquaporins belonging to the PIP2 group, both in higher plants and more primitive plants.

5 In an embodiment, a number of PIP2 sequences, from *Spinacia oleracea*, *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* or *Physcomitrella patens* are provided as SEQ ID NO: 4 to SEQ ID NO: 30.

Another embodiment relates to a method of obtaining constitutively open aquaporin. Said method comprises the step of adding a sequence having at least 90 %, 10 such as at least 95, 97 or 99%, sequence homology with SEQ ID NO: 3, to the C-terminus of a plasma membrane aquaporin. Preferably, said plasma membrane aquaporin belongs to the phylogenetic group PIP2.

Another embodiment relates to a constitutively open aquaporin comprising a C-terminal His-tag having at least 90 %, such as at least 95, 97 or 99%, sequence 15 homology with SEQ ID NO: 3.

Another embodiment relates to a membrane comprising a constitutively open aquaporin as disclosed herein.

According to an embodiment, sequence homology, i.e. sequence identity, is intended to mean the alignment sequence homology obtained by use of the BLAST- 20 algorithm using default settings.

Although the present invention has been described above with reference to specific embodiments, it is not intended to be limited to the specific form set forth herein. Rather, the invention is limited only by the accompanying claims and, other 25 embodiments than the specific above are equally possible within the scope of these appended claims.

In an embodiment, the membrane according to embodiments of the invention is incorporated into a device for purifying water. Said device comprises an inlet connected to a first chamber, for receiving dirty water, and an outlet connected to a second chamber for dispensing clean water. A water purifying membrane, comprising the 30 membrane according to embodiments of the invention, is arranged in between the first and the second chamber, so that water may pass from the first to the second chamber. The properties of the water purifying membrane and the membrane will be easily appreciated by a person skilled in the art, such as in view of US 7,857,978 B2, US 2009/0007555 A1, EP 1 885 477 B1, or Helix Nielsen, Anal Bioanal Chem (2009) 35 395:697–718.

In an embodiment, use of the membrane according to embodiments or the device, for purifying water is provided.

The purifying may be according to a method, comprising the steps of: providing a membrane according to embodiments; and passing water to be purified over
5 said membrane in order to provide purified water.

In the claims, the term "comprises/comprising" does not exclude the presence of other elements or steps. Furthermore, although individually listed, a plurality of means, elements or method steps may be implemented by e.g. a single unit. Additionally, although individual features may be included in different claims, these
10 may possibly advantageously be combined, and the inclusion in different claims does not imply that a combination of features is not feasible and/or advantageous. In addition, singular references do not exclude a plurality. The terms "a", "an", "first", "second" etc do not preclude a plurality. Reference signs in the claims are provided merely as a clarifying example and shall not be construed as limiting the scope of the
15 claims in any way.

Claims

1. A membrane for water purification comprising a constitutively open His-tagged aquaporin.

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2. The membrane according to claim 1, wherein the constitutively open His-tagged aquaporin has at least 90 %, such as at least 95, 97 or 99%, sequence homology with SEQ ID NO: 2.

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3. The membrane according to claim 1, comprising a constitutively open His-tagged aquaporin having amino acid sequence according to SEQ ID NO: 2.

4. The membrane according to claim 1, wherein the aquaporin originates from *Spinacia oleracea*, *Arabidopsis thaliana*, *Zea mays*, *Oryza sativa* or

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Physcomitrella patens.

5. The membrane according to claim 4, wherein the aquaporin comprises a sequence having at least 90 %, such as at least 95, 97 or 99%, sequence homology with any of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, or SEQ ID NO: 30.

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6. The membrane according to any of claims 1, 4 or 5, wherein the aquaporin comprises a C-terminal His-tag having at least 90 %, such as at least 95, 97 or 99%, sequence homology with SEQ ID NO: 3.

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7. The membrane according to claim 4, wherein the aquaporin comprises the sequence according to SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26,

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SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, or SEQ ID NO: 30, and a C-terminal His-tag having according to SEQ ID NO: 3.

5 8. A device for purifying water, comprising an inlet connected to a first chamber, for receiving dirty water, and an outlet connected to a second chamber for dispensing clean water, and a water purifying membrane, comprising the membrane according to any of claims 1 to 7, arranged in between the first and the second chamber.

10 9. Use of the membrane, according to any one of the claims 1 to 7, or a device according to claim 8, for purifying water.

10. A method for producing a membrane according to any of claims 1 to 7, comprising the steps of:

- 15 - producing a His-tagged aquaporin, which is constitutively open; and
- incorporating the aquaporin into a membrane.

11. A method of purifying water comprising the steps of:

- 20 - providing a membrane according to any of the claims 1 to 7; and
- passing water to be purified over said membrane in order to provide purified water.