

Hydroxy Artificial Water Channels Enable Ultrasensitive Reverse Osmosis Membranes for N-Nitrosamine Removal in Water Reuse

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The manuscript entitled “Hydroxy artificial water channels enable ultrasensitive reverse osmosis membrane for effective water reuse” provides valuable insights into the effects of the incorporation of hydroxy artificial water channels on RO membrane surfaces on their selectivity. However, the presentation of the results needs to be streamlined and the discussion needs bit more clarification and justification. Moreover, it often looks like that the authors jump to their conclusions without providing enough evidence. Based on these reasons, I do not recommend this manuscript for publication in Nature Communications.

Specific comments:

1. Title: Although this study has focused on the improved rejection of N-nitrosamines by RO membranes via incorporating hydroxy artificial water channels, the importance of N-nitrosamine removal is missing in the title of the manuscript.
2. There are a lot of grammatical errors and many expressions, including sentences, verb tenses, adverbs, and basic vocabulary, are awkward. I would suggest the authors to take a thorough review and editing of the manuscript by a native English speaker.
3. Abstract: The abstract should concisely state the main objectives of your research, principal results, and major conclusions. However, the authors just this displayed the reasons why they tried to develop ultrasensitive RO membranes using hydroxy artificial water channels for NDMA removal in the first 4 – 5 sentences. The authors are strongly advised to clarify the major conclusions drawn from their experiments and modeling.
4. Abstract: Please avoid to use personal pronouns (e.g., we, us, our, and etc.) in the main text to achieve more objective writing, unless the topic asks you to share your own experience.
5. Introduction: Although RO membranes have known to be ineffective for NDMA removal, NDMA exists in wastewater at very low levels. There is no reason to improve the selectivity of RO membranes by incorporating artificial water channels (AWCs), which might increase their manufacturing costs. Furthermore, even though nanochannels could facilitate the rejection of low molecular organic compounds, their installation on RO membrane surfaces might lead to a more severe membrane fouling. The authors should justify the trade-off relationship between the rejection of low molecular organic compounds and the formation of membrane fouling through incorporating artificial water channels on RO membrane surfaces.
6. Introduction: The necessity and novelty of this study have not been well described and the authors did not conduct literature review properly on the topic of the manuscript.
7. SI: The authors have not provided adequate references for the preparation and synthesis of nanochannels and RO membranes and etc.
8. This manuscript has not been adequately organized yet. It was difficult to follow. For better understanding, Results and Discussion sections have to be re-organized completely.
9. Characterizations of OH AWCs and AWCs: Although the authors characterized AWCs-incorporated RO membranes they synthesized using NMR, SEM, FTIR, and TEM, there was no evidence capable of visually identifying the installation of nanochannels on RO membrane surfaces. They just discussed on the qualitative analysis results obtained from the characterization of AWCs-incorporated RO membranes. I do not understand why they reached the conclusion that the

incorporation of AWCs made nanochannels on RO membrane surfaces. For validating this, the authors should provide information on the diameter of the AWCs.

10. Ultra-high separation performance of AWCs: Similarly, with providing any direct evidence for changing the pore size of RO membranes via incorporating nano-scale AWCs, how the authors reached conclusion that size exclusion played a crucial role in the removal of NaCl in this study? In addition, nobody assesses the performances of RO membranes at 7.8 bar! What about the charge properties of the tested N-nitrosamines? Although N-nitrosamines are generally uncharged at neutral pH conditions, the authors should explain thoroughly how they draw their conclusions.

11. Structural rigidity of AWCs: If swelling occurred, the mass or thickness of RO membranes should be changed. Did the authors confirm it?

12. Visualization of water molecules transport sites: What was the size of GNPs? The particle size must play a critical role in the distribution of GNPs on AWCs-incorporated RO membranes.

13. Inhibition of channel formation reveals the critical role of nanopores in OH AWCs: It was impossible to identify directly the self-assembly of nonporous SiO₂ and OUA (SiO₂@OUA) inhibit the formation of OH AWCs on RO membrane surfaces. Through qualitative analyses of OH AWCs-incorporated RO membranes, I wonder how the authors reached that conclusion.

Reviewer #2

(Remarks to the Author)

The authors of the manuscript titled "Hydroxy Artificial Water Channels Enable Ultrasensitive Reverse Osmosis Membrane for Effective Water Reuse" proposed new artificial water channel based RO membranes by incorporating hydroxy artificial water channels into polyamide (PA) thin-film composite (TFC) membranes prepared by interfacial polymerization (IP). The authors used hydroxy water channels (HWC) that Mihail and coworkers developed in 2021 (Huang LB, et al. Hydroxy channels—adaptive pathways for selective water cluster permeation. 547 Journal of the American Chemical Society 143, 4224-4233 (2021)) to make artificial water channel membranes (AWCMs). The authors fabricated few membranes with water channel contents and tested the membranes' water permeance (A value), NaCl rejection (%) and permeance (B value), and N-nitrosodimethylamine (NDMA) rejection and permeance (B value) properties. Those values were compared to the commercial and previously reported membranes to present AWCs' good separation performance. The experimental techniques and analysis such as TEM, FT-IR, Raman, MD simulations were performed to support the role of AWCs in the membranes in terms of increasing membrane separation performance. While those experiments partially support the author's argument, for me, it seems that several, but most critical logics are based on speculations and lack scientific evidence. Also, importantly, the similar concept of incorporating artificial water channels into PA membranes have been reported pretty much recently (Biomimetic artificial water channel membranes for enhanced desalination. Nat. Nanotechnol. 16, 190, (2021), Tunable membranes incorporating artificial water channels for high-performance brackish/low-salinity water reverse osmosis desalination, PNAS,

<https://doi.org/10.1073/pnas.2022200118>, (2021), Selectivity of artificial water channel-polyamide composite membranes toward inorganic contaminants, J. Membr. Sci. (2022), <https://www.sciencedirect.com/science/article/abs/pii/S0376738822007645>, review on artificial water channels incorporated polyamide membranes for water purification: Transport mechanisms and performance, Water Research, (2023), <https://www.sciencedirect.com/science/article/abs/pii/S0043135423012149>, Seamless incorporation of artificial water channels in defect-free polyamide membrane for desalination of brackish water, Nat. Commun. (2025), <https://www.nature.com/articles/s41467-025-59726-x>), and I am not sure whether this strategy of simply mixing artificial water channels into PA membranes have any unique scientific insights anymore or not. I would like to suggest major revision for this manuscript before recommending publication of this manuscript in Nature Communications.

Comment #1: The significance of this manuscript appears to lie in the molecular design of AWCs, which can be incorporated into PA layers, functioning as efficient water channels that selectively permeate water over salts. However, I remain unconvinced by this interpretation. My specific concerns are outlined below:

(1) First of all, I was embarrassed that, in the manuscript, I couldn't find the chemical structure of the HAW molecules at all... There is only awkward schematic chemical structure in Fig. 1, even without information on how this structure can form artificial water channels. I understand this structure was previously published in 2021 (even by the other research group), not all potential readers would know well about this biomimetic water channels and artificial water channel based membrane research fields. I would recommend the authors to provide those basic information.

(2) In original research paper that developed hydroxy water channels, the supramolecular hydroxy artificial water channel structures were determined via single-crystal analysis. Is there any experimental evidence confirming that these supramolecular channels structures are maintained within the PA matrix? Given that the single-crystal structure is known, the authors could compare the simulated PXRD pattern with the 1D XRD spectra or so, to check whether there is any overlapping diffraction peaks, which may indicate the retention of AWC structures within the PA layers. Alternatively, although challenging, since the authors suggest several spots in SEM images as artificial water channel aggregates, performing electron diffraction on these spots in TEM could help verify whether AWCs are incorporated into the PA matrix with intact supramolecular structure.

(3) Several experimental results have been included to show the presence of water channels in the membranes such as WAXS, SEM, Raman, FT-IR etc. But I think those are the results that support the existence of octyl-ureido-polyol (1-(1,3-79 dihydroxypropan-2-yl)-3-octylurea molecules, not self-assembled water channels. For example, in Fig. 2 A-C, those look just hydroxy-based molecules aggregates, not precisely and molecularly assembled water channel structures. In Fig. 2G, even though the authors argued that these data support the existence of 3.5Å pore sizes, I am not sure that amorphous diffraction peaks can be interpreted into such way the authors suggested. This result may challenge the author's interpretation because, continued from the above comments, if there were actually the self-assembled water channel structures, I would expect more clear diffraction peaks coming from the water channel structures.

(4) In Fig. 1B, is there any experimental results to support the H-bond formation between water channel molecules and MPDs? Or is it just suggesting one possibility? I am curious whether such a well aligned structures by H-bonds between hydroxy molecules and MPDs are maintained inside PA layer or not.

Comment #2: In the page 11, line 194, is the statement about the stability supported by any results?

Comment #3: In Fig. 4, I am not sure that the GNP clusters the evidence of water permeation spots. In general, GNPs are very prone to aggregate and those particles are stabilized by some surfactants. Hydroxy water channel molecules are amphiphilic and can serve as surfactant as well, probably just replacing the original surfactant and induce GNP aggregation or cluster formation near the spot where hydroxy water channels exist.

Comment #4: In the page 19, line 344, the authors claimed that this manuscript is the first to elucidate the mechanism by which AWCs enhance the performance of RO membranes. I would suggest reviewing some references I added in the first paragraph of this comment file.

Comment #5: In MD simulation part, how the authors constructed the water channel structure model? For performing MD simulations, the most important and challenging part may how to construct molecular structures that represent the real chemical structure of the water channels or membrane matrix. I tried to find out the details how the authors constructed these OH AWCs, the PA matrix, and the AWCs, but couldn't find out. Please clarify it and suggest that these structure are well representing the actual microstructures of the OH AWCs, PA matrix, and AWCs.

Reviewer #3

(Remarks to the Author)

"Please see attached."

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This manuscript has been well corrected and easy to follow. After reviewing the manuscript, I consider it is of enough quality to be published in Nature Communications.

Reviewer #2

(Remarks to the Author)

I appreciate the authors' efforts in revising the manuscript and preparing a detailed point-by-point rebuttal. The revised version is more polished in language and better organized than the original submission. The added experiments on fouling, high-salinity stability, and the SiO₂@OUA (channel inhibition system) certainly enrich the dataset. However, the core scientific concerns I had in the first round review process remain unresolved. First of all, the central mechanism that hydroxyl water channels (OH AWCs) act as the primary mass transfer channels within the polyamide (PA) layer is still not convincingly demonstrated and relies vastly on indirect evidence. Also, the novelty of the work is still limited relative to the rapidly growing literature on AWC-PA composite membranes and NDMA rejection. Several additional claims such as "excellent antifouling performance" and "first to elucidate the mechanisms by which AWCs enhance RO performance", remain overstated relative to the presented evidence. Because these points concern the main claims of the paper, I do not think they can be addressed with further revision process. Several detailed comments are as follows.

Comment #1: Evidence that OH AWCs are the "primary mass transfer channels" is still not compelling. The revised manuscript repeatedly and very strongly states that OH AWCs "serve as the primary mass transfer channels" and "govern the overall performance" of the membranes throughout the whole manuscript. While the authors have added more data such as GNP deposition patterns, Raman mapping of water distribution, SiO₂@OUA channel inhibition, and MD simulations, each suggested evidence is still indirect and can also be explained by more conventional changes in PA morphologies and free volume. For example, even though the authors claims that GNP clustering occurs near OH-AWCs, but there is no direct experimental evidence demonstrating spatial co-localization between OH-AWC domains and GNP deposition. All presented data (GNP patterns, flux differences, PA morphology changes) are indirect and can be explained by alternative factors such as local PA thickness, roughness, charge distribution, or free volume variation. Without direct co-mapping of AWC distribution and GNP deposition, the claim that GNP clusters uniquely mark the presence of AWCs is unsupported. Also, the Raman mapping does not provide any direct evidence of the spatial distribution of OH-AWCs as well. The measured OH-stretching signal merely reflects local water content and cannot be uniquely attributed to AWC domains. Considering the spatial resolution (>500 nm) relative to the AWC particle size (~15–24 nm), the Raman hotspots cannot represent AWC

locations. Thus, the assertion that Raman data undoubtedly indicate AWC-rich regions is not convincing to me.

Comment #2: Continued from the above comment, for the SiO₂@OUA channel inhibition experiments, inhibiting AWC self-assembly by co-assembling SiO₂ with OUA and then observing decreased performance (AWCM-0.25I) is interpreted as evidence that AWCs are the dominant transport pathways. However, the SiO₂@OUA aggregates introduce additional inorganic domains and also could alter the effective PA microstructure and cross-linking. These changes alone could decrease permeability and selectivity, independently of any loss of channel structures.

Comment #3: The MD simulations treat idealized OH AWCs and PA matrices separately and compare water density, diffusion coefficient, MSD, and energy barriers. However, these simulations effectively describe isolated “model” channels, not the actual composite thin-film structure formed by IP with 15–24 nm AWC particles embedded in a ~100–200 nm PA layer. There is no direct correlation that the simulated channel environment corresponds quantitatively to the real composite structure. As such, the MD results support the possibility that OH AWCs could be good transport pathways, but they do not prove that, in the real membrane, they are the dominant transport channels.

Comment #4: Most importantly, I think that this manuscript's novelty is limited relative to recent AWC-PA composite research. The revised introduction acknowledges that AWCs have been incorporated into PA RO membranes previously and cites several key works, as I also pointed out in the previous review round. However, the manuscript still positions itself as (1) elucidating for the first time the role of AWCs as primary mass transfer channels, and (2) the first to elucidate the mechanisms by which AWCs enhance the performance of RO membranes. In my perspective, these overstate the novelty. Several recent studies (some already cited in the rebuttal and I also commented before) have incorporated synthetic or biomimetic channels into PA membranes, discussed transport mechanisms and selectivity enhancements, and used simulations and advanced characterization to rationalize performance. In this context, the present manuscript appears as one more implementation of a similar concept (embedding a specific OH AWC into a PA layer) with performance in a comparable range, plus an extensive but still indirect mechanistic story. The incremental distinction—emphasizing NDMA and describing OH AWCs as primary mass transfer channels—does not seem sufficient to justify publication in a high-impact research journal.

Reviewer #3

(Remarks to the Author)

The authors have made commendable efforts in addressing the major concerns raised in the first round, and the revised manuscript reflects significant improvement. However, I would like to raise a point regarding the terminology used in the XRD analysis. The authors state in line 160: "Compared with the pristine RO membrane, the AWCM exhibited a red shift in the diffraction peak (Supplementary Fig. S20), which was attributed to the incorporation of OH AWCs into the PA layer." The use of the term “red shift” in the context is non-standard and potentially confusing, as it is typically reserved for optical spectroscopy. I suggest the authors revise this sentence to reflect standard terminology.

Reviewer #4

(Remarks to the Author)

This work demonstrates a polyamide membrane incorporated with hydroxyl artificial water channels (OH-AWCs) for the effective removal of N-nitrosodimethylamine (NDMA). While the separation performance is impressive, the mechanistic evidence supporting water transport through the OH-AWCs remains insufficient. The primary concerns, also noted by other reviewers, center on the validation of the proposed water transport pathway and the demonstration of novelty relative to other AWCs. To address these issues and meet the high standard of Nature Communications, I recommend the authors provide additional experimental and theoretical evidence:

1. Elucidating the water transport pathway through OH-AWCs:

The current Raman mapping data lack the spatial resolution to conclusively demonstrate water movement through the channels. Although control experiments offer indirect support, direct evidence is needed. A possible approach would be to employ isotopic labeling. By permeating deuterated water (D₂O) through the membrane and tracking its distribution, the primary transport pathway could be visualized. For instance, D₂O exhibits a characteristic IR peak near 2540 cm⁻¹ (Chem. Commun., 2015, 51, 3251-3254), which could be mapped using IR spectroscopic microscopy or related techniques. If D₂O is found to co-localize with the OH-AWCs rather than the bulk polyamide matrix, this would provide strong direct evidence for channel-mediated transport.

2. Substantiating the novelty of OH-AWCs:

The manuscript's central claim hinges on the unique efficacy of the hydroxyl-aligned channels. To better underscore this novelty, I recommend incorporating a comparative study. The authors could fabricate PA membranes with other, well-characterized AWCs and evaluate their performance alongside the OH-AWC variant. Additionally, molecular dynamics (MD) simulations comparing water transport, hydrogen-bonding dynamics, and rejection across different AWC types would greatly strengthen the argument. Such a combined experimental and theoretical comparison would not only clarify the advantage of OH-AWCs but also offer valuable design principles for future channel-based membranes.

Version 2:

Reviewer comments:

Reviewer #4

(Remarks to the Author)

I appreciate the authors' efforts to accept my suggestions on the IR mapping to track the water pathway. Now, the mapping in Fig. 5 J-K have shown the spatial correlation between the artificial channel and water pathway. I believe it can solve the related concerns raised by the other reviewers. My only suggestion is to improve the Fig. 5H, in particular at D₂O region on the following points:

1. Signal-to-noise ratio (SNR): They may extend the accumulation times for spectra collection, or reduce the thickness of membrane to suppress the signal from PA matrix. They may also convert the transmittance to absorbance unite if the SNR appears better.
2. Controlling baseline: The author can also add the IR spectra of pure PA matrix to check if there is an interference from PA matrix.
3. Experimental parameters: More details on the IR mapping can be added in the method section to establish an example for the other investigators on artificioal water channel. For instance, the intruement brand, detector, exposure/accumulation time, object etc.

Version 3:

Reviewer comments:

Reviewer #4

(Remarks to the Author)

All my concerns have been solved. Therefore, I recommentd this manuscript should be accepted.

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Response to Reviewer Comments and List of Changes Made in the Manuscript

Reviewer #1:

The manuscript entitled “Hydroxy artificial water channels enable ultraselective reverse osmosis membrane for effective water reuse” provides valuable insights into the effects of the incorporation of hydroxy artificial water channels on RO membrane surfaces on their selectivity. However, the presentation of the results needs to be streamlined and the discussion needs bit more clarification and justification. Moreover, it often looks like that the authors jump to their conclusions without providing enough evidence. Based on these reasons, I do not recommend this manuscript for publication in Nature Communications.

Our response: Thanks for your comments. In response to your comments and constructive suggestions, we have carefully incorporated them into the revised manuscript. Detailed point-by-point responses are provided below.

1. Title: *Although this study has focused on the improved rejection of N-nitrosamines by RO membranes via incorporating hydroxy artificial water channels, the importance of N-nitrosamine removal is missing in the title of the manuscript.*

Our response: Thanks for the incisive suggestion. According to your suggestion, we have modified the title in the revised manuscript as follows:

“Hydroxy Artificial Water Channels Enable Ultraselective Reverse Osmosis Membranes for Enhanced N-Nitrosamine Removal in Water Reuse”

2. *There are a lot of grammatical errors and many expressions, including sentences, verb tenses, adverbs, and basic vocabulary, are awkward. I would suggest the authors to take a thorough review and editing of the manuscript by a native English speaker.*

Our response: Thanks for the valuable suggestion. In accordance with your suggestion, we invited a native English speaker to thoroughly review and edit the manuscript. Relevant descriptions have been provided in the revised manuscript.

Lines 26-33 on Page 2 in the revised manuscript.

“OH AWCs serve as the primary mass transfer channels, enabling the membrane to deliver outstanding desalination performance ($99.2 \pm 0.3\%$), an exceptional NDMA rejection ($93.9 \pm 0.1\%$) that surpasses the existing upper bound of permselectivity, and an ultrahigh pure water permeance ($4.5 \pm 0.2 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1} \cdot \text{bar}^{-1}$) that exceeds that of state-of-the-art RO membranes. Within the OH AWCs, the hydrogen bonds formed between water molecules and -OH resulted in a short residence time and a high jump frequency of water molecules, enabling the formation of dynamic and continuous OH-H₂O networks that facilitated rapid water movement.”

Lines 76-81 on Page 4 in the revised manuscript.

“Consequently, AWCs could emerge as the primary mass transfer channels in RO membranes, dictating the overall separation performance. The potential role of AWCs as the primary mass transfer channels is elucidated for the first time, which could facilitate water molecule transport while inhibiting solute diffusion, thereby breaking through the upper bound of RO membrane performance and achieving simultaneous enhancement of water permeance and NDMA rejection.”

Lines 85-89 on Pages 4-5 in the revised manuscript.

“Herein, hydroxy artificial water channels (OH AWCs, sub-nanopore sizes ~ 3.5 Å) with excellent water/solute selectivity³⁶ were first self-assembled from octyl-ureido-polyol (1-(1,3-dihydroxypropan-2-yl)-3-octylurea (OUA)) (**Fig. 1A**), and subsequently incorporated into the PA layer of RO membranes (referred to as AWCM) using interfacial polymerization (IP) (**Fig. 1B**) to investigate the role of OH AWCs in the mass transfer process.”

Lines 89-93 on Page 5 in the revised manuscript.

“Visualizing water molecule permeation sites, inhibiting the self-assembly of OH AWCs, regulating the PA pores, and conducting molecular dynamics (MD) simulations revealed that OH AWCs functioned as the primary mass transfer channels (**Fig. 1C** and **Fig. 1D**). The study elucidates the mechanism by which AWCs enhance RO membrane performance, offering important guidance for the development of high-performance AWCs-based RO membranes.”

Lines 121-122 on Page 7 in the revised manuscript.

“Dynamic light scattering (DLS) was employed to investigate the colloidal aggregates formed by OH AWCs in various solutions.”

Lines 143-144 on Page 8 in the revised manuscript.

“Morphological and structural characterizations were further employed to probe whether OH AWCs were integrated into the PA layer.”

Lines 149-151 on Page 8 in the revised manuscript.

“Raman spectroscopy revealed the characteristic peaks of both OH AWCs and PA on the surface of the AWCM (**Supplementary Figs. S16-S17**), confirming the successful embedment of OH AWCs into the PA layer of the RO membrane.”

Lines 156-158 on Pages 8-9 in the revised manuscript.

“The O/N ratio of the AWCM decreased with increasing OH AWCs content, possibly because the increased cross-linking degree of the PA (which leads to a decrease in the O/N ratio) offset the enhancement in O/N ratio resulting from the incorporation of OH AWCs (**Supplementary Fig. S19**).”

Lines 171-172 on Page 9 in the revised manuscript.

“The separation performance of AWCM membranes was evaluated to verify that incorporating OH AWCs enhances RO membrane performance.”

Lines 200-202 on Page 11 in the revised manuscript.

“The effective removal of micropollutants, particularly the carcinogenic disinfection byproduct NDMA commonly found in the treatment of drinking water, domestic wastewater, industrial wastewater^{11, 12}, is of critical importance for water reuse safety and human health.”

Lines 205-207 on Page 11 in the revised manuscript.

“However, AWCM-0.25 exhibited excellent NDMA rejection of $93.9 \pm 0.1\%$ (**Fig. 3C**), corresponding to a 6.4-fold reduction in the permeate NDMA concentration compared with AWCM-0.”

Lines 209-213 on Page 11 in the revised manuscript.

“In addition to NDMA, AWCM-0.25 also exhibited excellent rejection of five other environmentally relevant nitrosamine compounds^{10,18}, including *N*-nitrosomethylethylamine (NMEA, $98.5 \pm 0.2\%$), *N*-nitrosopyrrolidine (NPYR, $99.5 \pm 0.1\%$), *N*-nitrosodiethylamine (NDEA, $99.4 \pm 0.2\%$), *N*-nitrosopiperidine (NPIP, $99.6 \pm 0.1\%$), and *N*-nitrosomorpholine (NMOR, $99.7 \pm 0.1\%$).”

Lines 250-251 on Page 13 in the revised manuscript.

“Gold nanoparticles (GNPs) filtration experiments and Raman mapping of the water distribution on the AWCM surface were adopted to analyze the interaction sites between water and the membrane.”

Lines 277-280 on Page 14 in the revised manuscript.

“Based on the self-assembly principle of SiO₂ and organic compounds⁴⁵, the self-assembly of nonporous SiO₂ and OUA (SiO₂@OUA) was conducted to inhibit the formation of OH AWCs, followed by an evaluation of the separation performance of the SiO₂@OUA modified RO membrane (referred to as AWCM-0.25I, with I standing for inhibited channel formation).”

Lines 300-301 on Page 15 in the revised manuscript.

“Furthermore, OH AWCs were confirmed as the primary mass transfer channels by adjusting the cross-linking degree of the PA layer (regulating PA pores) (**Fig. 5D**).”

Lines 313-316 on Page 16 in the revised manuscript.

“The results indicated that OH AWCs, serving as the primary mass transfer channels, exhibited high selectivity toward water molecules, enabling their preferential and rapid diffusion through the channels and thus mitigating the adverse effect of the increased cross-linking degree of the PA layer on water molecules transport.”

Lines 334-337 on Page 17 in the revised manuscript.

“When water molecules were transported in the AWCM, their density (**Fig. 6A**), diffusion coefficient (**Fig. 6B**), and mean squared displacement (MSD) (**Fig. 6C**) in the OH AWCs were significantly higher than in the PA matrix, confirming that the OH AWCs served as the primary mass transfer channels facilitating water transport.”

Lines 340-344 on Page 17 in the revised manuscript.

“MSD results also demonstrated that OH AWCs, serving as the primary mass transfer channels, enabled faster water diffusion, which was a key factor underlying the ultrahigh water permeance of the AWCM. During water transport in the AWCM, water molecules preferentially permeated through the OH AWCs, primarily due to the significantly lower permeation barrier of OH AWCs (1.7 eV) compared to that of the PA matrix (4.4 eV) (**Fig. 6F**).”

Lines 359-361 on Page 19 in the revised manuscript.

“As water molecules can act as both H-bonds donors and acceptors, they form OH-H₂O networks with the abundant -OH groups in the OH AWCs during transport³⁶, thereby creating continuous, low-energy pathways that account for their rapid permeation through the channels.”

Lines 373-377 on Pages 19-20 in the revised manuscript.

“Additionally, compared with the PA matrix (2.7 eV for NaCl and 2.4 eV for NDMA), NaCl and NDMA encountered greater difficulty passing through the OH AWCs (3.3 eV for NaCl and 5.3 eV for NDMA) due to their higher cross-channel barriers (**Fig. 6K** and **Fig. 6L**), indicating that the OH AWCs, as primary mass transfer channels, governed the overall performance of the AWCM and provided guidance for the design of high-performance AWCs-based RO membranes.”

Lines 384-385 on Page 20 in the revised manuscript.

“OH AWCs retain their intact structures within the PA matrix and act as the primary mass transfer channels, thereby enhancing the performance of RO membranes.”

Lines 395-398 on Page 20 in the revised manuscript.

“Furthermore, the confinement effect of the OH AWCs, serving as the primary mass transfer channels in the AWCM, imposed higher energy barriers for solute transport, hindering their diffusion and thereby enabling the AWCM to achieve excellent NaCl and NDMA rejection.”

Lines 400-403 on Page 21 in the revised manuscript.

“The study is the first to elucidate the mechanisms by which AWCs, serving as the primary mass transfer channels, enhance the performance of RO membranes, providing crucial guidance for the development of high-performance AWCs-based RO membranes.”

Lines 482-487 on Page 24 in the revised manuscript.

“Developing RO membranes with stable performance under high-salinity conditions is of great importance. Given the exceptional stability of OH AWCs as the primary mass transfer channels in RO membranes, they hold significant potential for enhancing membrane stability in such environments. Therefore, the variation in the separation performance of the AWCM membranes was examined under different NaCl concentrations (40 bar; 2, 15, 25, and 40 g/L) to investigate the contribution of the rigidity of OH AWCs to the overall membrane performance.”

Lines 512-514 on Page 25 in the revised manuscript.

“The GNPs-deposited membranes were dried under vacuum and subsequently characterized by SEM to investigate the distribution of GNPs on the membrane surface, thereby identifying the water transport sites.”

Lines 522-523 on Page 26 in the revised manuscript.

“Eventually, the distribution of water on the membrane surface was analyzed to determine the water transport sites.”

3. Abstract: The abstract should concisely state the main objectives of your research, principal results, and major conclusions. However, the authors just this displayed the reasons why they tried to develop ultraselective RO membranes using hydroxy artificial water channels for NDMA removal in the first 4-5 sentences. The authors are strongly advised to clarify the major conclusions drawn from their experiments and modeling.

Our response: Thanks for the incisive suggestion. According to your suggestion, we have revised the abstract in the revised manuscript to highlight the major conclusions, as detailed below:

“Reverse osmosis (RO) is a leading technology of water reuse for addressing global water scarcity. However, current RO membranes often struggle to effectively reject certain harmful small molecules in water and wastewater, such as *N*-nitrosodimethylamine (NDMA). Herein, inspired by the channel proteins in cell membranes, an ultrasensitive RO membrane (AWCM) was developed by incorporating hydroxy artificial water channels (OH AWCs) into the polyamide (PA) rejection layer. OH AWCs serve as the primary mass transfer channels, enabling the membrane to deliver outstanding desalination performance ($99.2 \pm 0.3\%$), an exceptional NDMA rejection ($93.9 \pm 0.1\%$) that surpasses the existing upper bound of permselectivity, and an ultrahigh pure water permeance ($4.5 \pm 0.2 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1} \cdot \text{bar}^{-1}$) that exceeds that of state-of-the-art RO membranes. Within the OH AWCs, the hydrogen bonds formed between water molecules and -OH resulted in a short residence time and a high jump frequency of water molecules, enabling the formation of dynamic and continuous OH-H₂O networks that facilitated rapid water movement. In contrast, NaCl and NDMA faced high trans-channel energy barriers, which significantly hindered their transport through the OH AWCs. The resulting AWCM membrane outperforms prevailing RO membranes and offers valuable insights for the design of next-generation RO membranes.”

4. *Abstract: Please avoid to use personal pronouns (e.g., we, us, our, and etc.) in the main text to achieve more objective writing, unless the topic asks you to share your own experience.*

Our response: Thanks for the incisive suggestion. In accordance with your suggestion, we have removed personal pronouns such as “we”, “us”, and “our” from the manuscript. Relevant descriptions have been provided in the revised manuscript.

Lines 24-26 on Pages 2 in the revised manuscript.

“Herein, inspired by the channel proteins in cell membranes, an ultrasensitive RO membrane (AWCM) is developed by incorporating hydroxy artificial water channels (OH AWCs) into the polyamide (PA) rejection layer.”

Lines 85-89 on Pages 4-5 in the revised manuscript.

“Herein, hydroxy artificial water channels (OH AWCs, sub-nanopore sizes $\sim 3.5 \text{ \AA}$) with excellent water/solute selectivity³⁶ were first self-assembled from octyl-ureido-polyol (1-(1,3-dihydroxypropan-2-yl)-3-octylurea (OUA)) (**Fig. 1A**), and subsequently incorporated into the PA layer of RO membranes (referred to as AWCM) using interfacial polymerization (IP) (**Fig. 1B**) to investigate the role of OH AWCs in the mass transfer process.”

Lines 89-93 on Page 5 in the revised manuscript.

“Visualizing water molecule permeation sites, inhibiting the self-assembly of OH AWCs, regulating the PA pores, and conducting molecular dynamics (MD) simulations revealed that OH AWCs functioned as the primary mass transfer channels (**Fig. 1C** and **Fig. 1D**). The study

elucidates the mechanism by which AWCs enhance RO membrane performance, offering important guidance for the development of high-performance AWCs-based RO membranes.”

Lines 121-122 on Page 7 in the revised manuscript.

“Dynamic light scattering (DLS) was employed to investigate the colloidal aggregates formed by OH AWCs in various solutions.”

Lines 143-144 on Page 8 in the revised manuscript.

“Morphological and structural characterizations were further employed to probe whether OH AWCs were integrated into the PA layer.”

Lines 250-251 on Page 13 in the revised manuscript.

“Gold nanoparticles (GNPs) filtration experiments and Raman mapping of the water distribution on the AWCM surface were adopted to analyze the interaction sites between water and the membrane.”

Lines 277-280 on Page 14 in the revised manuscript.

“Based on the self-assembly principle of SiO₂ and organic compounds⁴⁵, the self-assembly of nonporous SiO₂ and OUA (SiO₂@OUA) was conducted to inhibit the formation of OH AWCs, followed by an evaluation of the separation performance of the SiO₂@OUA modified RO membrane (referred to as AWCM-0.25I, with I standing for inhibited channel formation).”

Lines 300-301 on Page 15 in the revised manuscript.

“Furthermore, OH AWCs were confirmed as the primary mass transfer channels by adjusting the cross-linking degree of the PA layer (regulating PA pores) (**Fig. 5D**).”

Lines 382-384 on Page 20 in the revised manuscript.

“In this study, OH AWCs modified RO membranes were fabricated that concurrently enhanced membrane separation performance and anti-swelling performance in high-salinity environments.”

Lines 400-403 on Page 21 in the revised manuscript.

“The study is the first to elucidate the mechanisms by which AWCs, serving as the primary mass transfer channels, enhance the performance of RO membranes, providing crucial guidance for the development of high-performance AWCs-based RO membranes.”

5. Introduction: Although RO membranes have known to be ineffective for NDMA removal, NDMA exists in wastewater at very low levels. There is no reason to improve

the selectivity of RO membranes by incorporating artificial water channels (AWCs), which might increase their manufacturing costs. Furthermore, even though nanochannels could facilitate the rejection of low molecular organic compounds, their installation on RO membrane surfaces might lead to a more severe membrane fouling. The authors should justify the trade-off relationship between the rejection of low molecular organic compounds and the formation of membrane fouling through incorporating artificial water channels on RO membrane surfaces.

Our response: Thanks for the incisive suggestion. We would like to reply to it in a point-by-point manner.

(i) *Although RO membranes have known to be ineffective for NDMA removal, NDMA exists in wastewater at very low levels. There is no reason to improve the selectivity of RO membranes by incorporating artificial water channels (AWCs), which might increase their manufacturing costs.*

Reply to point (i): Thanks for the suggestion. NDMA, a typical disinfection by-product commonly found in the treatment of drinking water, domestic wastewater, industrial wastewater, etc., is highly carcinogenic even at trace levels and poses a serious threat to human health (*Environmental Science & Technology*, 2018,52: 10215-10223; *Separation and Purification Technology*, 2012, 98: 503-515). In California, a notification level of 10 ng/L and a public health goal of 3 ng/L have been established for NDMA (*Water Research*, 2018, 131: 45-51). Therefore, developing PA RO membranes capable of efficiently removing NDMA is of great significance. At present, commercial RO membranes, originally designed for salt removal, typically exhibit limited NDMA rejection, with removal rates ranging from 30% to 80% at the laboratory scale and only 5% to 60% at the pilot and full scales, thereby necessitating additional energy-intensive treatment processes to eliminate NDMA from the RO permeate (*Science Advances*, 2022, 8: eabm4149; *Journal of Membrane Science*, 2018, 554: 301-308). Artificial water channels (AWCs), featuring superior water/solute selectivity, represent a cutting-edge strategy for enhancing RO membrane performance by serving as primary mass transfer channels when incorporated into the PA layer, enabling high water permeance and efficient NDMA removal. Relevant descriptions have been provided in the revised manuscript.

Lines 43-54 on Page 3 in the revised manuscript.

“Currently, existing polyamide (PA) RO membranes, primarily designed for salt removal^{7, 8}, typically achieve high NaCl rejection (>99%) but exhibit inadequate removal efficiency for certain harmful small molecules, such as *N*-nitrosodimethylamine (NDMA)^{9, 10}. NDMA, a typical disinfection by-product commonly found in the treatment of drinking water, domestic wastewater, industrial wastewater, etc.,^{11, 12} is highly carcinogenic even at trace levels and poses a serious threat to human health. In California, a notification level of 10 ng/L and a public health goal of 3 ng/L have been established for NDMA¹⁰. At present, commercial RO membranes demonstrate NDMA rejection rates ranging from 30% to 80% in laboratory-scale

tests and only 5% to 60% in pilot to full-scale systems^{13, 14}, requiring the additional implementation of energy-intensive processes to remove NDMA from the RO permeate^{15, 16}. Therefore, developing PA RO membranes capable of efficiently removing NDMA is of great significance for advancing water reuse and safeguarding public health.”

Lines 66-71 on Page 4 in the revised manuscript.

“Artificial water channels (AWCs) that are inspired from the channel proteins in cell membranes, featuring with Å-scale mass transfer channels and superior transport properties, exhibit higher water permeance-selectivity compared to RO PA layer^{23, 24, 25, 26, 27, 28}. Therefore, incorporating AWCs into the PA layer of RO membranes (AWCs-based RO membranes) represents a cutting-edge approach to surpassing the upper bound of RO membrane performance and holds great promise for the efficient removal of NDMA^{27,29,30}.”

(ii) Furthermore, even though nanochannels could facilitate the rejection of low molecular organic compounds, their installation on RO membrane surfaces might lead to a more severe membrane fouling.

Reply to point (ii): Thanks for the suggestion. We evaluated the resistance of AWCM membranes to humic acid (HA) fouling to assess the effect of OH AWCs on antifouling performance. The results indicated that the incorporation of OH AWCs into the PA layer of RO membranes not only enhanced the rejection of low molecular weight organic compounds but also imparted excellent antifouling performance to the membranes. Relevant descriptions have been provided in the revised manuscript.

Lines 213-217 on Page 11 in the revised manuscript.

“Moreover, AWCM-0.25 exhibited superior resistance to humic acid (HA) fouling compared with AWCM-0 (**Supplementary Fig. S25**), indicating that the incorporation of OH AWCs into RO membranes not only enhanced the rejection of neutral small-molecule pollutants but also imparted excellent antifouling performance to the membranes.”

Lines 475-477 on Page 24 in the revised manuscript.

“In addition, a mixed solution containing 100 mg/L HA and 2000 mg/L NaCl was used to evaluate the antifouling performance of the membranes (initial flux of $\sim 35 \text{ L m}^{-2} \text{ h}^{-1}$, pH=7, 25°C).”

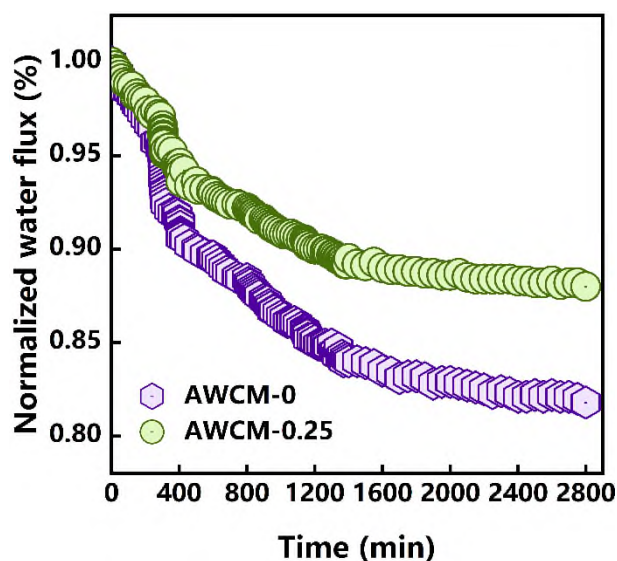


Fig. S25. Normalized water flux as a function of time for AWCM-0 and AWCM-0.25 membranes during the antifouling experiments. The flux decline curves represent averaged data from duplicate fouling tests. A mixed solution containing 100 mg/L HA and 2000 mg/L NaCl was used to evaluate the antifouling performance of the membranes (initial flux of $\sim 35 \text{ L m}^{-2} \text{ h}^{-1}$, pH=7, 25°C).

(iii) The authors should justify the trade-off relationship between the rejection of low molecular organic compounds and the formation of membrane fouling through incorporating artificial water channels on RO membrane surfaces.

Reply to point (iii): Thanks for the suggestion. By testing the NDMA rejection of AWCM-0 and AWCM-0.25 membranes under the same degree of fouling, we revealed a trade-off between the rejection of low-molecular-weight organic compounds and membrane fouling. Relevant descriptions have been added to the revised manuscript.

Lines 217-222 on Pages 11-12 in the revised manuscript.

“Notably, when the membranes were fouled to the same degree (flux decline of $\sim 10\%$), NDMA rejection decreased for both AWCM-0 and AWCM-0.25 (**Supplementary Fig. S26**). Compared with their pristine counterparts, AWCM-0.25 ($\sim 9.3\%$ decrease in NDMA rejection) exhibited a smaller decline than AWCM-0 ($\sim 20.9\%$ decrease in NDMA rejection), indicating that the incorporation of OH AWCs into the RO membrane could partially mitigate the adverse effects of membrane fouling on pollutant rejection.”

Lines 477-479 on Page 24 in the revised manuscript.

“The rejection of NDMA by AWCM-0 and AWCM-0.25 membranes under the same degree of fouling (flux decline of $\sim 10\%$) was further investigated.”

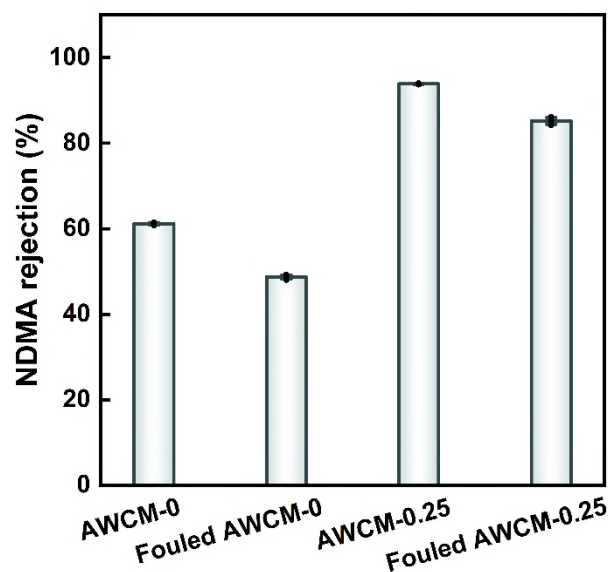


Fig. S26. NDMA rejection of AWCm-0 and AWCm-0.25 membranes before fouling and under the same degree of HA fouling. Error bars represent the s.d. ($n = 3$), and data are presented as mean $\pm$ s.d.

6. *Introduction: The necessity and novelty of this study have not been well described and the authors did not conduct literature review properly on the topic of the manuscript.*

Our response: Thanks for the valuable suggestion. We have added additional literature reviews to declare the necessity and novelty of this study. The necessity and novelty of this study are as follows: i) NDMA, a typical disinfection by-product commonly found in the treatment of drinking water, domestic wastewater, industrial wastewater, etc., is highly carcinogenic even at trace levels and poses a serious threat to human health (*Environmental Science & Technology*, 2018, 52: 10215-10223; *Separation and Purification Technology*, 2012, 98: 503-515). In California, a notification level of 10 ng/L and a public health goal of 3 ng/L have been established for NDMA (*Water Research*, 2018, 131: 45-51). Therefore, the removal of NDMA from water is of great importance for water reuse and public health. ii) Commercial RO membranes, originally designed for salt removal, typically exhibit limited NDMA rejection, necessitating additional energy-intensive treatment processes to eliminate NDMA from the RO permeate (*Science Advances*, 2022, 8: eabm4149; *Journal of Membrane Science*, 2018, 554: 301-308). Artificial water channels (AWCs), featuring superior water/solute selectivity, represent a cutting-edge strategy to enhance RO membrane performance through incorporation into the PA layer, enabling the resulting membranes to surpass the upper bounds of conventional RO membranes (*Chemical Society Reviews*, 2022, 51: 4537-4582) (*Chemical Society Reviews*, 2022, 51: 4537-4582). Furthermore, incorporating AWCs into the PA layer is straightforward and requires no major changes to existing industrial fabrication protocols. iii) For the first time, this study reveals that AWCs incorporated within the PA rejection layer act as primary transport channels enhancing RO membrane performance and provides

mechanistic insights into water and solute diffusion inside the AWCs. Relevant descriptions have been provided in the revised manuscript.

Lines 43-54 on Page 3 in the revised manuscript.

“Currently, existing polyamide (PA) RO membranes, primarily designed for salt removal^{7, 8}, typically achieve high NaCl rejection (>99%) but exhibit inadequate removal efficiency for certain harmful small molecules such as *N*-nitrosodimethylamine (NDMA)^{9, 10}. NDMA, a typical disinfection by-product commonly found in the treatment of drinking water, domestic wastewater, industrial wastewater, etc.,^{11, 12} is highly carcinogenic even at trace levels and poses a serious threat to human health. In California, a notification level of 10 ng/L and a public health goal of 3 ng/L have been established for NDMA¹⁰. At present, commercial RO membranes demonstrate NDMA rejection rates ranging from 30% to 80% in laboratory-scale tests and only 5% to 60% in pilot to full-scale systems^{13, 14}, requiring the additional implementation of energy-intensive processes to remove NDMA from the RO permeate^{15, 16}. Therefore, developing PA RO membranes capable of efficiently removing NDMA is of great significance for advancing water reuse and safeguarding public health.”

Lines 66-71 on Page 4 in the revised manuscript.

“Artificial water channels (AWCs) that are inspired from the channel proteins in cell membranes, featuring with Å-scale mass transfer channels and superior transport properties, exhibit higher water permeance-selectivity compared to RO PA layer^{23, 24, 25, 26, 27, 28}. Therefore, incorporating AWCs into the PA layer of RO membranes (AWCs-based RO membranes) is a cutting-edge approach to surpassing the upper bound of RO membrane performance and holds great promise for the efficient removal of NDMA^{27, 29, 30}.”

Lines 71-75 on Page 4 in the revised manuscript.

“For example, incorporating AWCs (specifically, hexylureido-ethyl-imidazole) into the PA layer improved the separation performance of the RO membranes³¹. Nevertheless, the mysterious role of AWCs when coexisting with the PA rejection layer, whose nanochannels may play a critical role in mass transfer and largely determine the overall membrane performance, remains unexplored.”

Lines 76-84 on Page 4 in the revised manuscript.

“Consequently, AWCs could emerge as the primary mass transfer channels in RO membranes, dictating the overall separation performance. The potential role of AWCs as the primary mass transfer channels is elucidated for the first time, which could facilitate water molecule transport while inhibiting solute diffusion, thereby breaking through the upper bound of RO membrane performance and achieving simultaneous enhancement of water permeance and NDMA rejection. Furthermore, the incorporation of AWCs into the PA layer is relatively straightforward and does not require substantial modifications to the established industrial

fabrication protocols for commercial RO membranes, offering great potential for large-scale implementation.”

7. *SI: The authors have not provided adequate references for the preparation and synthesis of nanochannels and RO membranes and etc.*

Our response: Thanks for the incisive suggestion. Relevant references on the preparation and synthesis of OH AWCs and RO membranes have been added to the supporting information (**Text S2** and **Text S3**). The specific references are listed below:

- “6. Song X, et al. Intrinsic Nanoscale Structure of Thin Film Composite Polyamide Membranes: Connectivity, Defects, and Structure–Property Correlation. *Environmental Science & Technology* 54, 3559-3569 (2020).
7. Di Vincenzo M, et al. Biomimetic artificial water channel membranes for enhanced desalination. *Nature Nanotechnology* 16, 190-196 (2021).
8. Shi M, Wang Z, Zhao S, Wang J, Zhang P, Cao X. A novel pathway for high performance RO membrane: Preparing active layer with decreased thickness and enhanced compactness by incorporating tannic acid into the support. *Journal of Membrane Science* 555, 157-168 (2018).
9. Gan Q, et al. Demystifying the Role of Surfactant in Tailoring Polyamide Morphology for Enhanced Reverse Osmosis Performance: Mechanistic Insights and Environmental Implications. *Environmental Science & Technology* 57, 1819-1827 (2023).
10. Huang L-B, et al. Hydroxy Channels–Adaptive Pathways for Selective Water Cluster Permeation. *Journal of the American Chemical Society* 143, 4224-4233 (2021).
11. Licsandru E, et al. Salt-Excluding Artificial Water Channels Exhibiting Enhanced Dipolar Water and Proton Translocation. *Journal of the American Chemical Society* 138, 5403-5409 (2016).
12. Murail S, Vasiliu T, Neamtu A, Barboiu M, Sterpone F, Baaden M. Water permeation across artificial I-quartet membrane channels: from structure to disorder. *Faraday Discussions* 209, 125-148 (2018).”

8. *This manuscript has not been adequately organized yet. It was difficult to follow. For better understanding, Results and Discussion sections have to be re-organized completely.*

Our response: Thanks for the incisive suggestion. We have reorganized the results and discussion sections. The logical structure of the results and discussion is as follows: i) the structural characteristics of OH AWCs and RO membranes incorporated with OH AWCs were first characterized, confirming that the OH AWCs retained their intact structures within the PA layer; ii) subsequently, the water permeance, NaCl rejection, NDMA rejection, and stability of the RO membranes were evaluated, confirming that the incorporation of OH AWCs enabled superior performance of the RO membranes; iii) through MD simulations, water permeation visualization, inhibition of OH AWCs self-assembly, and regulation of PA pore structures, OH AWCs within the PA layer were confirmed as the primary mass transfer channels, dictating the overall separation performance of

the membranes; iv) a comprehensive analysis of the mass transfer mechanisms of water molecules and solutes within OH AWCs elucidated the mechanisms by which OH AWCs serve as the primary mass transfer channels to enhance the performance of RO membranes.

9. Characterizations of OH AWCs and AWCM: Although the authors characterized AWCs-incorporated RO membranes they synthesized using NMR, SEM, FTIR, and TEM, there was no evidence capable of visually identifying the installation of nanochannels on RO membrane surfaces. They just discussed on the qualitative analysis results obtained from the characterization of AWCs-incorporated RO membranes. I do not understand why they reached the conclusion that the incorporation of AWCs made nanochannels on RO membrane surfaces. For validating this, the authors should provide information on the diameter of the AWCs.

Our response: Thanks for the incisive suggestion. We would like to respond to this suggestion in a point-by-point manner.

(i) Characterizations of OH AWCs and AWCM: Although the authors characterized AWCs-incorporated RO membranes they synthesized using NMR, SEM, FTIR, and TEM, there was no evidence capable of visually identifying the installation of nanochannels on RO membrane surfaces. They just discussed on the qualitative analysis results obtained from the characterization of AWCs-incorporated RO membranes. I do not understand why they reached the conclusion that the incorporation of AWCs made nanochannels on RO membrane surface.

Reply to point (i): Thanks for the suggestion. OH AWCs were incorporated throughout the entire PA matrix, forming nanochannels distributed across the matrix rather than only on the surface. These nanochannels are the intrinsic channels of OH AWCs, which act as the primary mass transfer channels in the mixed rejection layer of PA and OH AWCs. This is primarily because OH AWCs present a lower permeation barrier for water molecules than the PA layer, enabling preferential water permeation through their intrinsic channels. Therefore, proper distribution of OH AWCs within the PA matrix is crucial for effective nanochannel formation in the membranes. We now provide visual evidence confirming the successful distribution of OH AWCs within the PA matrix through surface images (**Fig. 2(I-L)**) and cross-sectional images (**Supplementary Fig. S21**) of the AWCM membrane. Relevant descriptions have been provided in the revised manuscript.

Lines 144-149 on Page 8 in the revised manuscript.

“Although the AWCM-0 (without OH AWCs), AWCM-0.10, AWCM-0.25, and AWCM-0.60 (AWCM-0.10, AWCM-0.25, and AWCM-0.60 represented RO membranes modified with OH AWCs self-assembled from 0.1 wt%, 0.25 wt%, and 0.6 wt% OUA, respectively) all exhibited a characteristic “ridge-and-valley” structure, numerous nanoparticles, namely OH AWCs, were interspersed within the structure of the AWCM-0.10, AWCM-0.25, and AWCM-0.60 membranes (**Fig. 2(I-L)**), indicating successful incorporation of OH AWCs into the PA layer.”

Lines 164-168 on Page 9 in the revised manuscript.

“Cross-sectional characterization of the AWC membranes further confirmed the successful distribution of OH AWCs within the PA matrix (**Supplementary Fig. S21(A–C)**). Moreover, OH AWCs retained well-defined lattice structures in the PA matrix (**Supplementary Fig. S21D**), indicating that they remained structurally intact and favoring their intrinsic nanochannels acting as the primary mass transfer pathways within the PA matrix.”

Lines 442-445 on Page 22 in the revised manuscript.

“The cross-section of AWC membranes was investigated by TEM. Membrane coupons were first embedded in epoxy resin, and an ultramicrotome equipped with a diamond knife was then used to slice the resin-embedded membranes to prepare TEM cross-sectional samples. The resulting samples were mounted onto carbon-coated TEM grids for further characterization.”

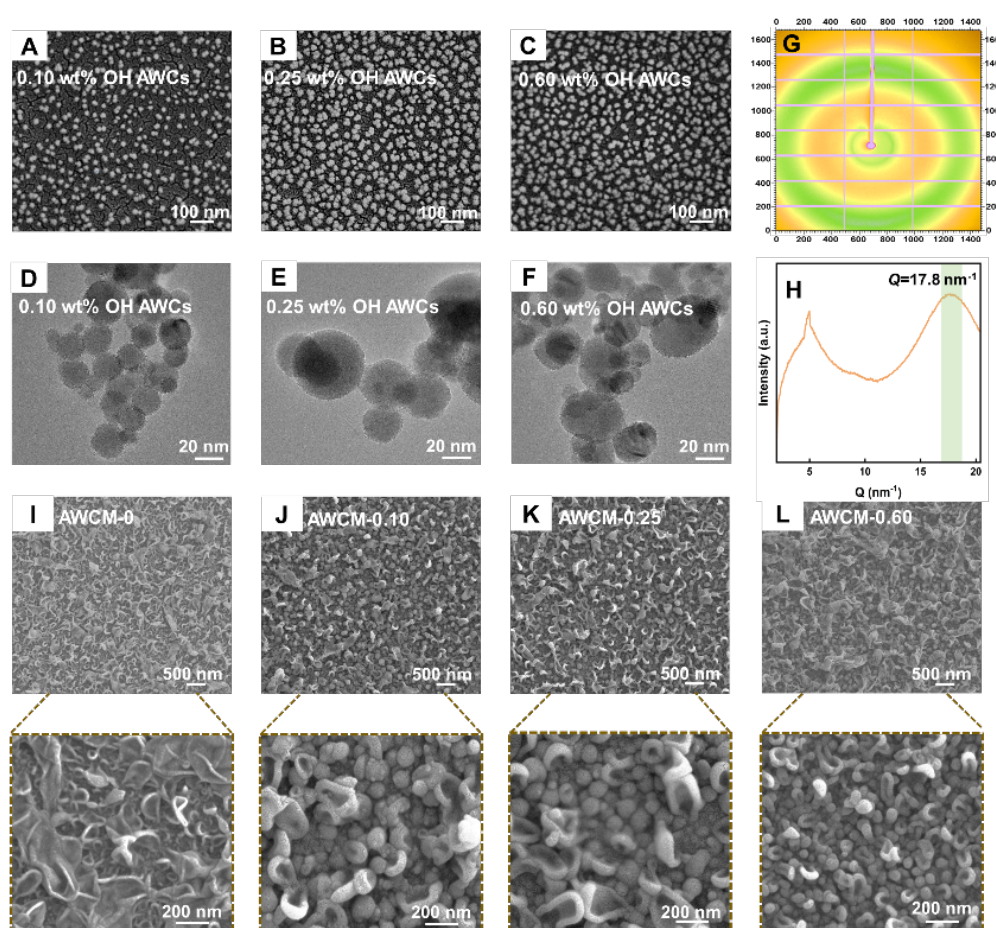


Fig. 2. Characterizations of OH AWCs and AWC membrane. (A–C) Scanning electron microscopy (SEM) images of OH AWCs. (D–F) Transmission electron microscope (TEM) images of OH AWCs. (G–H) The wide-angle X-ray scattering (WAXS) spectra of OH AWCs. The 1-(1,3-dihydroxypropan-2-yl)-3-octylurea at concentrations of 0.1 wt%, 0.25 wt%, and 0.6 wt% self-assembled into OH AWCs in a water/ethanol solution, then deposited onto the surface of polysulfone membranes and dropped onto a copper mesh for SEM and TEM characterization,

respectively. (I-L) Morphology of the AWCm-0 (without OH AWCs), AWCm-0.10, AWCm-0.25, and AWCm-0.60 membranes (AWCm-0.10, AWCm-0.25, and AWCm-0.60 represented RO membranes modified with OH AWCs self-assembled from 0.1 wt%, 0.25 wt%, and 0.6 wt% 1-(1,3-dihydroxypropan-2-yl)-3-octylurea, respectively).

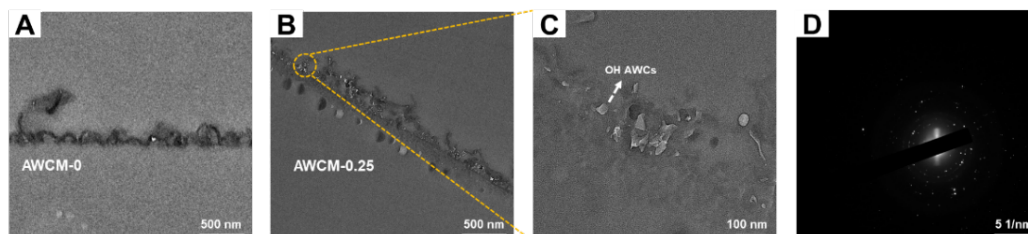


Fig. S21. Cross-sectional characterization of (A) AWCm-0 and (B) AWCm-0.25. (C) High-magnification TEM cross-section of AWCm-0.25. (D) Electron diffraction pattern of OH AWCs within the PA matrix.

(ii) For validating this, the authors should provide information on the diameter of the AWCs.

Reply to point (ii): Thanks for the suggestion. Based on TEM characterization, the average size of OH AWCs was measured using Nano Measurer 1.2 software. Relevant descriptions have been provided in the revised manuscript.

Lines 119-120 on Pages 6-7 in the revised manuscript.

“With the OH AWCs concentration increasing from 0.1 wt% to 0.6 wt%, the average size of individual OH AWCs increased from ~15.1 to ~24.1 nm (Supplementary Fig. S11).”

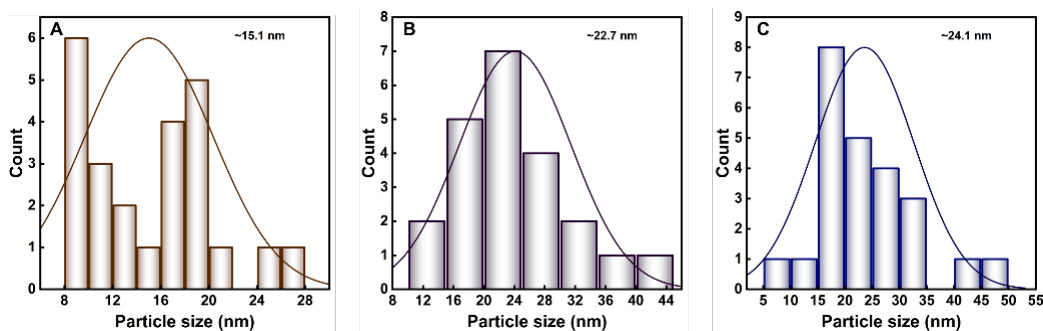


Fig. S11. Size distribution of OH AWCs at (A) 0.10 wt%, (B) 0.25 wt%, and (C) 0.60 wt%, calculated from TEM images using Nano Measurer 1.2 software.

10. Ultra-high separation performance of AWCm: Similarly, with providing any direct evidence for changing the pore size of RO membranes via incorporating nano-scale AWCs, how the authors reached conclusion that size exclusion played a crucial role in the removal of NaCl in this study? In addition, nobody assesses the performances of RO membranes at 7.8 bar! What about the charge properties of the tested N-nitrosamines? Although N-nitrosamines are generally uncharged at neutral pH conditions, the authors should explain thoroughly how they draw their conclusions.

Our response: Thanks for the incisive suggestion. In response to your suggestion, we have carefully incorporated them into the revised manuscript. Detailed point-by-point responses are provided below.

(i) Ultra-high separation performance of AWCm: Similarly, with providing any direct evidence for changing the pore size of RO membranes via incorporating nano-scale AWCs, how the authors reached conclusion that size exclusion played a crucial role in the removal of NaCl in this study?

Reply to point (i): Thanks for the suggestion. We now confirm through positron annihilation lifetime spectroscopy and neutral molecular probe experiments that incorporating OH AWCs into the PA layer of RO membranes enhances the size exclusion effect. Relevant descriptions have been provided in the revised manuscript.

Lines 176-178 on Page 9 in the revised manuscript.

“The superior NaCl rejection of AWCm-0.25 was attributed to enhanced size exclusion, as evidenced by the narrower pore size distribution (**Supplementary Fig. S22**) and improved neutral molecule rejection (**Supplementary Fig. S23**) compared with AWCm-0.”

Lines 457-458 on Page 23 in the revised manuscript.

“Positron annihilation lifetime spectroscopy (PALS, DPLS3000, ORTEC, USA) was employed to analyze the pore size distribution of the membranes.”

Lines 469-470 on Page 23 in the revised manuscript.

“Ethylene glycol (62 Da) was used as a neutral molecular probe (concentration: 40 mg/L) to analyze the size exclusion of the membranes.”

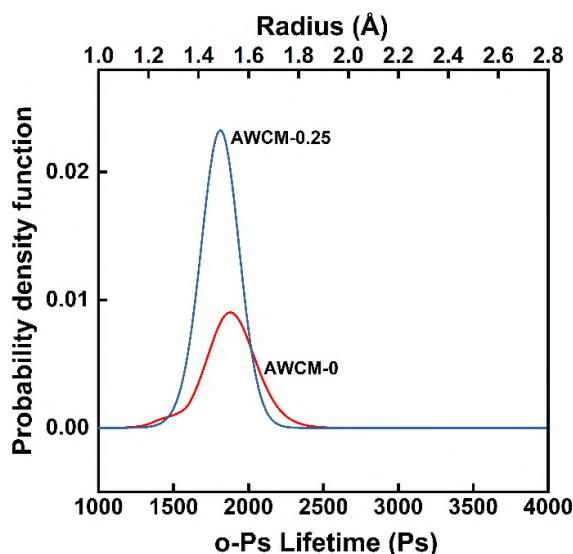


Fig. S22. The probability density distributions of pore sizes in AWCM-0 and AWCM-0.25 were obtained from the positron annihilation lifetime spectrum.

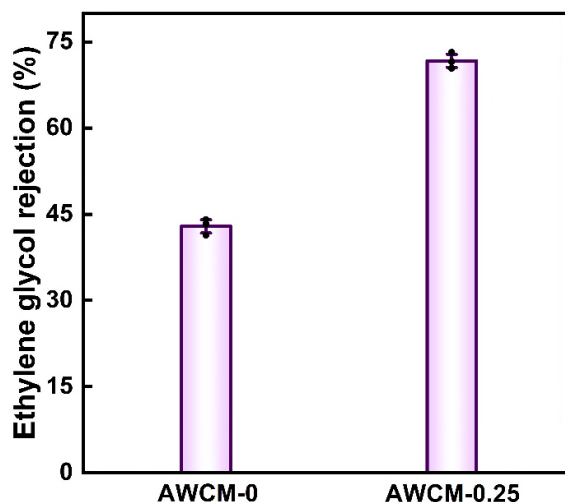


Fig. S23. Rejection of ethylene glycol by AWCM-0 and AWCM-0.25 membranes (concentration: 40 mg/L; applied pressure: 16 bar; temperature: 25°C). Error bars represent the s.d. ($n = 3$), and data are presented as mean $\pm$ s.d.

(ii) *In addition, nobody assesses the performances of RO membranes at 7.8 bar!*

Reply to point (ii): Thanks for the suggestion. In our study, the performance of RO membranes was tested at a pressure of 16 bar, not 7.8 bar. The value of 7.8 bar⁻¹ mentioned in the main text refers to the water/NaCl selectivity of the AWCM-0.25 membrane. As described in the main text:

Lines 182-183 on Page 10 in the revised manuscript.

“Compared to the AWCM-0 (~ 2.7 bar⁻¹), the water/NaCl selectivity of AWCM-0.25 (~ 7.8 bar⁻¹) was increased by $\sim 188.9\%$.”

(iii) *What about the charge properties of the tested N-nitrosamines? Although N-nitrosamines are generally uncharged at neutral pH conditions, the authors should explain thoroughly how they draw their conclusions.*

Reply to point (iii): Thanks for the suggestion. In our study, N-nitrosamine rejection by RO membranes was evaluated under neutral conditions. Previous studies (*Water Research*, 2018, 131: 45-51; *Separation and Purification Technology*, 2012, 98: 503-515) have shown that N-nitrosamines mainly exist in neutral molecular form at neutral pH. This is primarily attributed to the lack of strongly basic sites for protonation and the absence of dissociable acidic protons in N-nitrosamines (*Journal of organic chemistry*, 2021, 86(3): 2037-2057; *Toxicology and Applied Pharmacology*, 1975, 31: 325-351). Relevant descriptions have been provided in the revised manuscript.

Lines 56-58 on Page 3 in the revised manuscript.

“Under neutral conditions, NDMA remains uncharged primarily due to the absence of strongly basic protonation sites and dissociable acidic protons^{19, 20}.”

11. *Structural rigidity of AWCm: If swelling occurred, the mass or thickness of RO membranes should be changed. Did the authors confirm it?*

Our response: Thanks for the incisive suggestion. The PA layer thicknesses of the conventional RO membrane and the RO membrane incorporated with OH AWCs were measured before and after exposure to a high-salinity environment. The results showed that the PA layer of the conventional RO membrane became thicker after high salinity exposure, confirming that swelling occurred. Relevant descriptions have been provided in the revised manuscript.

Lines 228-231 on Page 12 in the revised manuscript.

“The decrease in apparent water permeance of the AWCm-0 membrane was attributed to the swelling of the PA layer^{41, 42}, as evidenced by a ~27.3% increase in PA layer thickness after exposure to a high-salinity environment (Supplementary Fig. S27).”

Lines 231-234 on Page 12 in the revised manuscript.

“Owing to no significant change in the PA layer thickness of the AWCm-0.25 membrane after exposure to a high-salinity environment (Supplementary Fig. S28), the decrease in apparent water permeance for the AWCm-0.25 membrane was attributed to the effects of concentration polarization and osmotic pressure.”

Lines 449-454 on Page 23 in the revised manuscript.

“Furthermore, AFM was employed to assess the apparent thickness of the PA layer of the AWCm membranes before and after exposure to a high-salinity environment. The membrane substrate was first dissolved in DMF, and the isolated PA layer was transferred onto a silicon wafer for thickness measurement by scanning the interface between the PA layer and the wafer surface. The PA layer on the wafer was then immersed in a 40 g/L NaCl solution for 24 h, after which its thickness was measured again.”

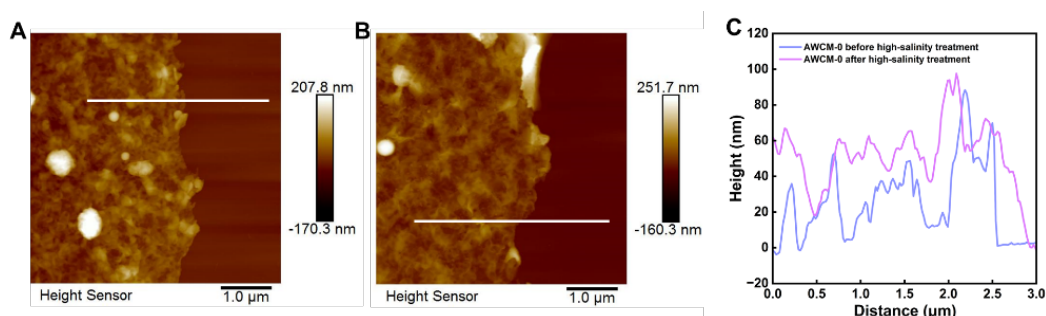


Fig. S27. AFM characterized the height profiles of the AWCM-0 membranes. (A-B) 2D images of AWCM-0 membranes before and after high-salinity treatment. (C) Height profiles of AWCM-0 membranes before and after high-salinity treatment.

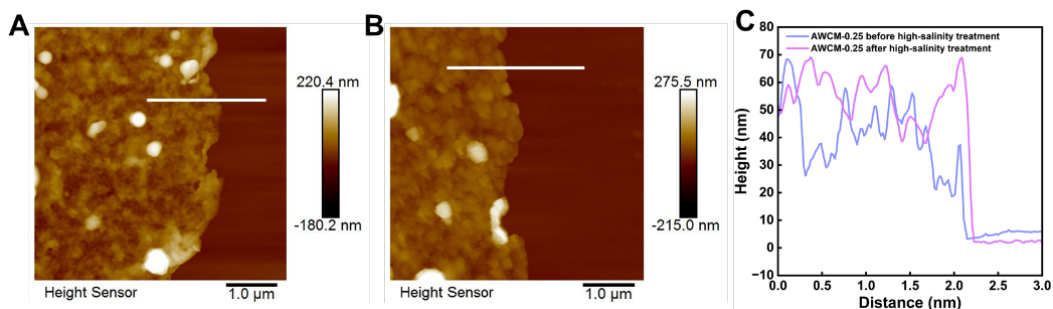


Fig. S28. AFM characterized the height profiles of the AWCM-0.25 membranes. (A-B) 2D images of AWCM-0.25 membranes before and after high-salinity treatment. (C) Height profiles of AWCM-0.25 membranes before and after high-salinity treatment.

12. *Visualization of water molecules transport sites: What was the size of GNPs? The particle size must play a critical role the distribution of GNPs on AWCs-incorporated RO membranes.*

Our response: Thanks for the suggestion. The size of GNPs is 5 nm. Visualizing water permeation sites through GNPs filtration experiments is an effective strategy (*Science*, 2018, 360: 518-521). Under neutral conditions, GNPs are negatively charged and exhibit surface charge behavior similar to that of RO membranes. For GNPs deposition to occur, the drag force must overcome the electrostatic repulsion between the GNPs and the membrane surface, and therefore the resulting spatial distribution of GNPs is primarily governed by the water transport pathways. Consequently, the deposition sites of GNPs correspond to the water permeation sites, providing direct visual evidence of water transport pathways. Relevant descriptions have been provided in the revised manuscript.

Lines 251-255 on Page 13 in the revised manuscript.

“Under neutral conditions, GNPs are negatively charged and exhibit similar surface charge behavior to RO membranes (**Supplementary Fig. S34**)^{43, 44}. For GNPs deposition to occur, the drag force must overcome the electrostatic repulsion between the GNPs and the membrane surface, and therefore the resulting spatial distribution of GNPs is primarily governed by the water transport pathways⁴³.”

Lines 418-420 on Page 21 in the revised manuscript.

“A citrate-stabilized dispersed GNPs solution (5 nm, 5×10^{13} particles/mL) for investigating the water transport pathways was supplied by BBI Solutions (UK) and stored at 4°C.”

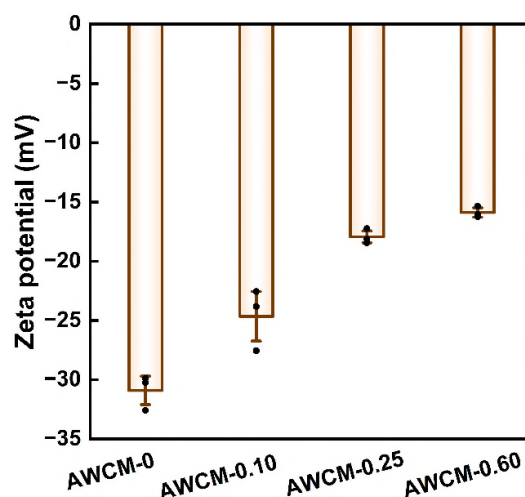


Fig. S34. The surface charge of AWCM-0, AWCM-0.10, AWCM-0.25, and AWCM-0.60 membranes. Error bars represent the s.d. ($n = 3$), and data are presented as mean values $\pm$ s.d.

13. Inhibition of channel formation reveals the critical role of nanopores in OH AWCs: It was impossible to identify directly the self-assembly of nonporous SiO₂ and OUA (SiO₂@OUA) inhibit the formation of OH AWCs on RO membrane surfaces. Through qualitative analyses of OH AWCs-incorporated RO membranes, I wonder how the authors reached that conclusion.

Our response: Thanks for the suggestion. We now demonstrate through MD simulations that SiO₂ can self-assemble with OUA through H-bonds and van der Waals interactions, inhibiting the formation of OH AWCs, confirming that the decline in the performance of the SiO₂@OUA modified RO membranes (AWCM-0.25I) was attributed to the insufficient quantity of available OH AWCs for mass transfer within the PA layer. Relevant descriptions have been provided in the revised manuscript.

Lines 280-285 on Pages 14-15 in the revised manuscript.

“MD simulations confirmed that SiO₂ and OUA can interact in water/ethanol solution (**Supplementary Figs. S36-S37**) and self-assemble into a stable aggregate (**Supplementary Fig. S38**), effectively inhibiting the formation of OH AWCs. The self-assembly of SiO₂ and OUA was mainly driven by H-bonds (formed between Si-OH groups and the -OH, -C=O, and -NH groups in the OUA structure) and van der Waals (vdW) interactions (arising from the alkyl chains of OUA and Si atoms) (**Fig. 5A** and **Supplementary Figs. S39-S40**).”

Lines 528-530 on Page 26 in the revised manuscript.

“In addition, MD simulations were used to confirm that SiO₂ and OUA could undergo a self-assembly process, and the detailed procedure was presented in **Supplementary Text S7**.”

Details of the simulation process of SiO₂ and OUA have been provided in the supporting information (**Text S7**) as follows:

“Nonporous SiO₂ and OUA molecules were drawn using chemical software. The molecular structures were optimized using the B3LYP functional of the density functional theory (DFT) method with the 6-31G(d) basis set in Gaussian 0. The optimized structures were then used to calculate the RESP charges of the repeating unit fragments via Multiwfn, and these charges were employed to construct the force-field parameters for the small molecules based on GAFF^{22, 23, 24}. The parameters for SiO₂ were adopted from literature-reported force fields²⁵. All simulations were performed using GROMACS 2023.2²⁶. The systems were parameterized with the AMBER14SB²⁷ force field to describe their overall properties. OUA and nonporous SiO₂ were added to three simulation boxes at a molar ratio of 77:10, along with ethanol at a volume ratio of 1:1 with water. The box dimensions were set such that the distance between the box boundaries and the contents was 15 Å, and the systems were solvated with TIP3P water molecules. Energy minimization was carried out using the steepest descent algorithm (1000 kJ/mol/nm), followed by a 0.5 ns NPT equilibration. Simulation parameters were set as follows: temperature was maintained at 298 K using the V-rescale²⁸ thermostat, and pressure was kept at 1 bar using the C-rescale barostat²⁹. Long-range van der Waals interactions were truncated at 10 Å, and dispersion corrections were applied using EnerPres. Trajectory snapshots were obtained using VMD 1.9.3, and detailed molecular interactions were visualized with PyMOL 2.5.0.”

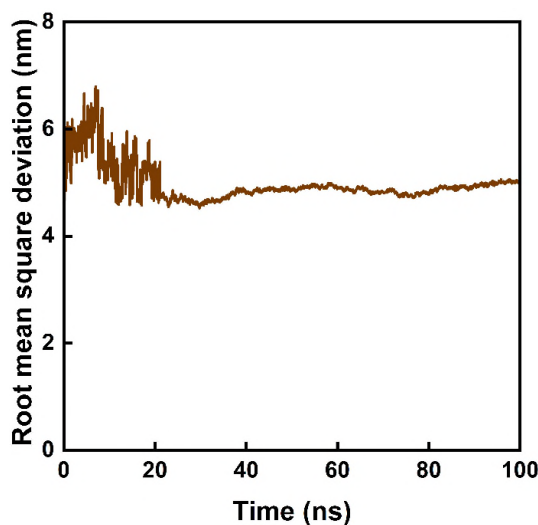


Fig. S36. Root mean square deviation of SiO₂ and OUA during the self-assembly simulation.

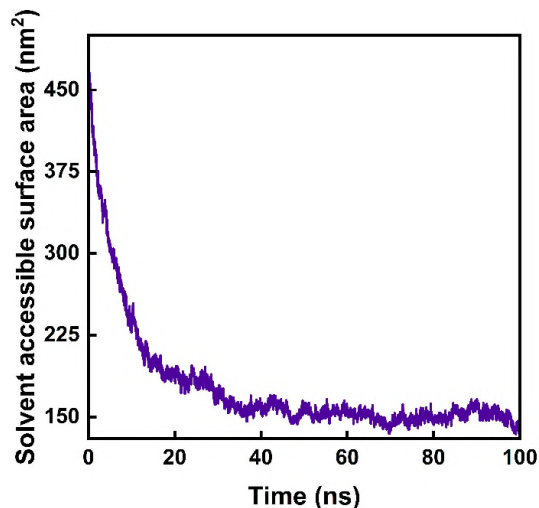


Fig. S37. Solvent accessible surface area of SiO₂ and OUA during the self-assembly simulation.

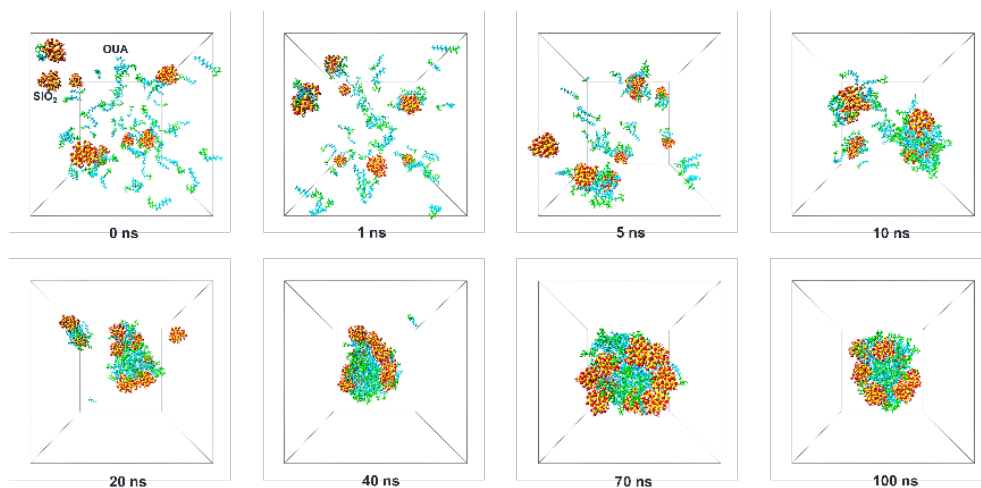


Fig. S38. Snapshots of the overall structures of SiO₂ and OUA self-assembly at different simulation times.

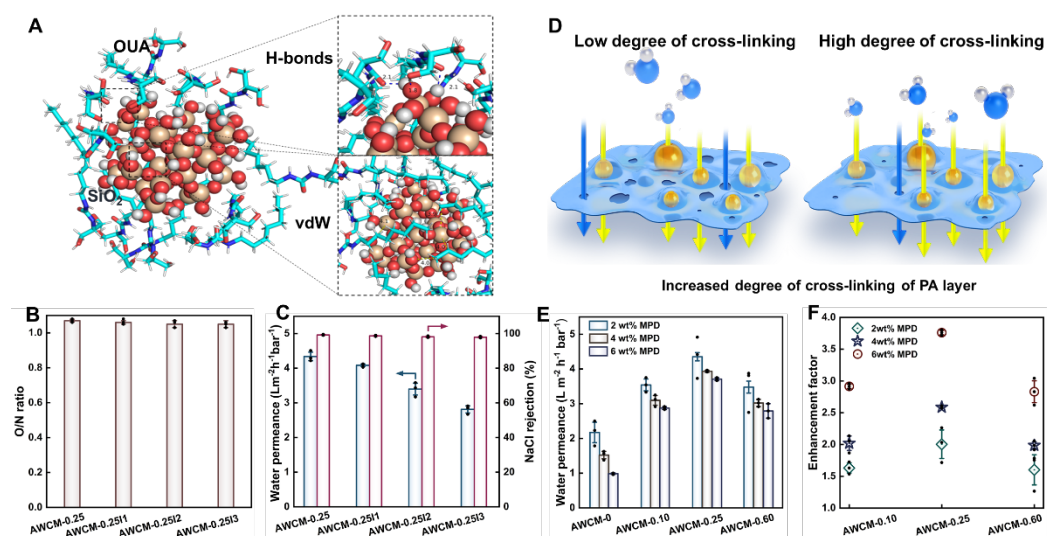


Fig. 5. Separation performance of the AWCM-0.25 and AWCM-0.25I (I standing for inhibited

channels formation) membrane. (A) The self-assembly of nonporous SiO₂ and OUA. (B) The O/N ratio of the AWCM-0.25, AWCM-0.25I1, AWCM-0.25I2, and AWCM-0.25I3 (AWCM-0.25, AWCM-0.25I1, AWCM-0.25I2, and AWCM-0.25I3 represent the SiO₂@OUA modified RO membranes assembled at SiO₂ and OUA mass ratios of 0, 1:4, 1:2, and 1:1, respectively). (C) Water permeance (NaCl solution) and NaCl rejection of the AWCM-0.25, AWCM-0.25I1, AWCM-0.25I2, and AWCM-0.25I3 membranes [NaCl (2000 mg/L), 16 bar, 25°C]. Membrane performance under different cross-linking degree. (D) Water transport behavior in AWCM with different cross-linking degree. (E) Water permeance (NaCl solution) of AWCM-0, AWCM-0.10, AWCM-0.25, and AWCM-0.60 membranes under different MPD concentrations. (F) The enhancement factor of water permeance for AWCM-0.10, AWCM-0.25 and AWCM-0.60 compared to the AWCM-0 under different MPD concentrations. Error bars in B-C and E-F represent the s.d. ($n = 3$) and data are presented as mean values $\pm$ s.d.

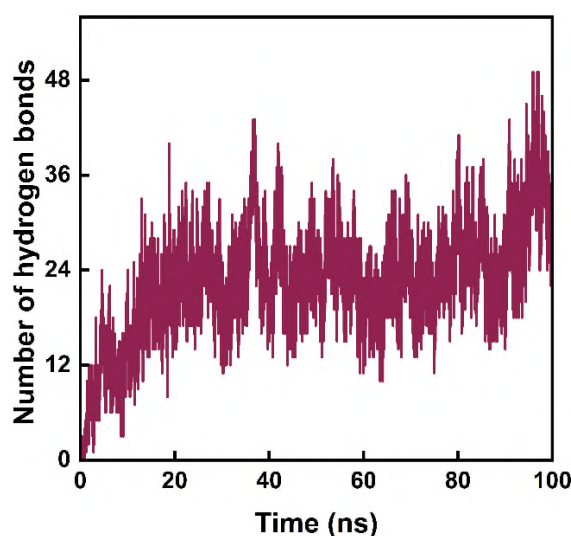


Fig. S39. Changes in the number of H-bonds between SiO₂ and OUA during the self-assembly simulation.

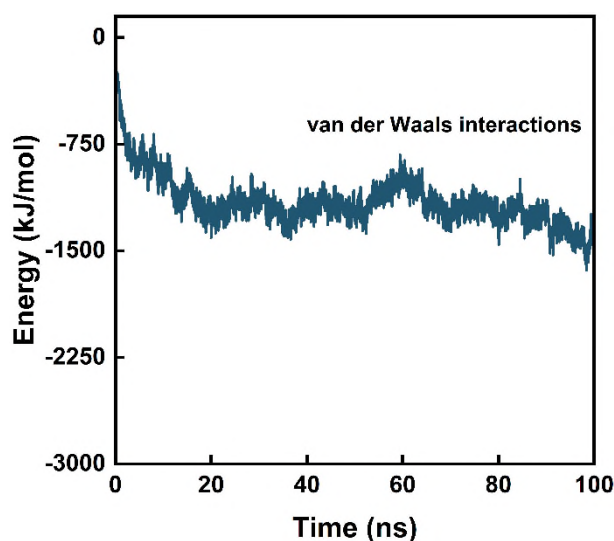


Fig. S40. Van der Waals interactions between SiO₂ and OUA during the self-assembly simulation.

Reviewer #2:

The authors of the manuscript titled “Hydroxy Artificial Water Channels Enable Ultraselective Reverse Osmosis Membrane for Effective Water Reuse” proposed new artificial water channel based RO membranes by incorporating hydroxy artificial water channels into polyamide (PA) thin-film composite (TFC) membranes prepared by interfacial polymerization (IP). The authors used hydroxy water channels (HWC) that Mihail and coworkers developed in 2021 (Huang LB, et al. Hydroxy channels–adaptive pathways for selective water cluster permeation. 547 Journal of the American Chemical Society 143, 4224-4233 (2021)) to make artificial water channel membranes (AWCMs). The authors fabricated few membranes with water channel contents and tested the membranes’ water permeance (A value), NaCl rejection (%) and permeance (B value), and N-nitrosodimethylamine (NDMA) rejection and permeance (B value) properties. Those values were compared to the commercial and previously reported membranes to present AWCMs’ good separation performance. The experimental techniques and analysis such as TEM, FT-IR, Raman, MD simulations were performed to support the role of AWCs in the membranes in terms of increasing membrane separation performance. While those experiments partially support the author’s argument, for me, it seems that several, but most critical logics are based on speculations and lack scientific evidence. Also, importantly, the similar concept of incorporating artificial water channels into PA membranes have been reported pretty much recently (Biomimetic artificial water channel membranes for enhanced desalination. Nat. Nanotechnol. 16, 190, (2021), Tunable membranes incorporating artificial water channels for high-performance brackish/low-salinity water reverse osmosis desalination, PNAS, <https://doi.org/10.1073/pnas.2022200118>, (2021), Selectivity of artificial water channel-polyamide composite membranes toward inorganic contaminants, J. Membr. Sci. (2022), <https://www.sciencedirect.com/science/article/abs/pii/S0376738822007645>, review on artificial water channels in incorporated polyamide membranes for water purification: Transport mechanisms and performance, Water Research, (2023), <https://www.sciencedirect.com/science/article/abs/pii/S0043135423012149>, Seamless incorporation of artificial water channels in defect-free polyamide membrane for desalination of brackish water, Nat. Commun. (2025), <https://www.nature.com/articles/s41467-025-59726-x>), and I am not sure whether this strategy of simply mixing artificial water channels into PA membranes have any unique scientific insights anymore or not. I would like to suggest major revision for this manuscript before recommending publication of this manuscript in Nature Communications.

Our response: Thanks for the incisive suggestion. We have carefully revised our manuscript based on the suggestions. Point-by-point responses are provided below.

(i) While those experiments partially support the author’s argument, for me, it seems that several, but most critical logics are based on speculations and lack scientific evidence.

Reply to point (i): Thanks for the suggestion. We have added further data to support our conclusions, as detailed in the point-by-point responses below.

(ii) Also, importantly, the similar concept of incorporating artificial water channels into PA membranes have been reported pretty much recently (Biomimetic artificial water channel membranes for enhanced desalination. *Nat. Nanotechnol.* 16, 190, (2021), Tunable membranes incorporating artificial water channels for high-performance brackish/low-salinity water reverse osmosis desalination, *PNAS*, <https://doi.org/10.1073/pnas.2022200118>, (2021), Selectivity of artificial water channel-polyamide composite membranes toward inorganic contaminants, *J. Membr. Sci.* (2022), <https://www.sciencedirect.com/science/article/abs/pii/S0376738822007645>, review on artificial water channels incorporated polyamide membranes for water purification: Transport mechanisms and performance, *Water Research*, (2023), <https://www.sciencedirect.com/science/article/abs/pii/S0043135423012149>, Seamless incorporation of artificial water channels in defect-free polyamide membrane for desalination of brackish water, *Nat. Commun.* (2025), <https://www.nature.com/articles/s41467-025-59726-x>), and I am not sure whether this strategy of simply mixing artificial water channels into PA membranes have any unique scientific insights anymore or not.

Reply to point (ii): Thanks for the suggestion. Although the above literature reported that incorporating AWCs into the PA layer of RO membranes can enhance membrane separation performance, they did not elucidate how AWCs participate in and govern the mass transfer process within the mixed rejection layer of AWCs and PA. Our study demonstrates that AWCs serve as the primary mass transfer channels in the mixed rejection layer of AWCs and PA, thereby determining the separation performance of the RO membrane and elucidating the distinct mass transfer mechanisms of water molecules and solutes within the AWCs. Furthermore, our study addresses the current limitation of the poor removal efficiency of the toxic small neutral molecular environmental pollutant NDMA by commercial RO membranes. At present, commercial RO membranes, originally designed for salt removal, typically exhibit limited NDMA rejection, with removal rates ranging from 30% to 80% at the laboratory scale and only 5% to 60% at the pilot and full scale, thereby necessitating additional energy-intensive treatment processes to eliminate NDMA from the RO permeate (*Science Advances*, 2022, 8: eabm4149; *Journal of Membrane Science*, 2018, 554: 301-308). In our study, OH AWCs were incorporated into the PA layer of RO membranes as primary mass transfer channels, significantly enhancing NDMA removal efficiency and surpassing the current water/NDMA selectivity upper bound. Relevant descriptions have been provided in the revised manuscript.

Lines 43-54 on Page 3 in the revised manuscript.

“Currently, existing polyamide (PA) RO membranes, primarily designed for salt removal^{7, 8}, typically achieve high NaCl rejection (>99%) but exhibit inadequate removal efficiency for

certain harmful small molecules such as *N*-nitrosodimethylamine (NDMA)^{9, 10}. NDMA, a typical disinfection by-product commonly found in the treatment of drinking water, domestic wastewater, industrial wastewater, etc.,^{11, 12} is highly carcinogenic even at trace levels and poses a serious threat to human health. In California, a notification level of 10 ng/L and a public health goal of 3 ng/L have been established for NDMA¹⁰. At present, commercial RO membranes demonstrate NDMA rejection rates ranging from 30% to 80% in laboratory-scale tests and only 5% to 60% in pilot to full-scale systems^{13, 14}, requiring the additional implementation of energy-intensive processes to remove NDMA from the RO permeate^{15, 16}. Therefore, developing PA RO membranes capable of efficiently removing NDMA is of great significance for advancing water reuse and safeguarding public health.”

Lines 72-75 on Page 4 in the revised manuscript.

“Nevertheless, the mysterious role of AWCs when coexisting with the PA rejection layer, whose nanochannels may play a critical role in mass transfer and largely determine the overall membrane performance, remains unexplored.”

Lines 76-81 on Page 4 in the revised manuscript.

“Consequently, AWCs could emerge as the primary mass transfer channels in RO membranes, dictating the overall separation performance. The potential role of AWCs as the primary mass transfer channels is elucidated for the first time, which could facilitate water molecule transport while inhibiting solute diffusion, thereby breaking through the upper bound of RO membrane performance and achieving simultaneous enhancement of water permeance and NDMA rejection.”

1: The significance of this manuscript appears to lie in the molecular design of AWCs, which can be incorporated into PA layers, functioning as efficient water channels that selectively permeate water over salts. However, I remain unconvinced by this interpretation. My specific concerns are outlined below:

(1) First of all, I was embarrassed that, in the manuscript, I couldn't find the chemical structure of the HAW molecules at all... There is only awkward schematic chemical structure in Fig. 1, even without information on how this structure can form artificial water channels. I understand this structure was previously published in 2021 (even by the other research group), not all potential readers would know well about this biomimetic water channels and artificial water channel based membrane research fields. I would recommend the authors to provide those basic information.

Our response: Thanks for the incisive suggestion. We have now provided the chemical structures of OH AWCs and their precursor OUA, along with a detailed description of the OH AWCs formation process. Relevant descriptions have been provided in the revised manuscript.

Lines 107-111 on Page 6 in the revised manuscript.

“The OUA molecule consists of a hydrophobic alkyl chain and a hydrophilic polyhydroxy head group connected by a urea linkage (-NH-CO-NH-) (**Supplementary Fig. S3**). The urea segment is capable of forming directional H-bonds, which drive the formation of an ordered channel structure (OH AWCs) through H-bonds interactions in water/ethanol solution (**Supplementary Fig. S3**).”

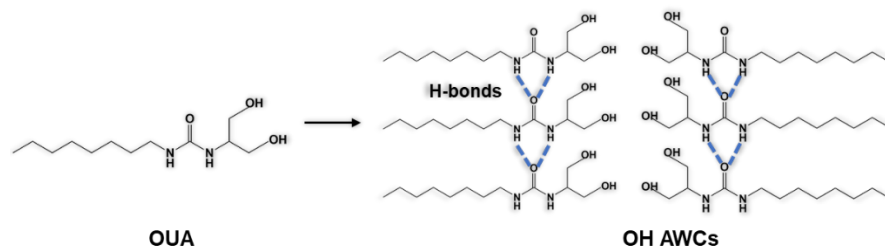


Fig. S3. Chemical structures of OUA and OH AWCs self-assembled from OUA.

(2) In original research paper that developed hydroxy water channels, the supramolecular hydroxy artificial water channel structures were determined via single-crystal analysis. Is there any experimental evidence confirming that these supramolecular channels structures are maintained within the PA matrix? Given that the single-crystal structure is known, the authors could compare the simulated PXRD pattern with the 1D XRD spectra or so, to check whether there is any overlapping diffraction peaks, which may indicate the retention of AWC structures within the PA layers. Alternatively, although challenging, since the authors suggest several spots in SEM images as artificial water channel aggregates, performing electron diffraction on these spots in TEM could help verify whether AWCs are incorporated into the PA matrix with intact supramolecular structure.

Our response: Thanks for the incisive suggestion. We now provide XRD patterns of OH AWCs and AWCM membranes, along with cross-sectional TEM images of AWCM and electron diffraction patterns of OH AWCs within AWCM, demonstrating that the OH AWCs largely retained their intact structures within the PA layer. Relevant descriptions have been provided in the revised manuscript.

Lines 158-168 on Page 9 in the revised manuscript.

“In addition, X-ray diffraction (XRD) characterization was performed to analyze the structure of OH AWCs within the PA layer. Compared with the pristine RO membrane, the AWCM exhibited a red shift in the diffraction peak (**Supplementary Fig. S20**), which was attributed to the incorporation of OH AWCs into the PA layer. Moreover, characteristic diffraction peaks of OH AWCs were detected on the surface of the AWCM membrane, indicating that OH AWCs retained their intrinsic structural features within the PA layer. Cross-sectional characterization of the AWCM membranes further confirmed the successful distribution of OH AWCs within the PA matrix (**Supplementary Fig. S21(A–C)**). Moreover, OH AWCs retained well-defined lattice structures in the PA matrix (**Supplementary Fig. S21D**), indicating that they remained structurally intact and favoring their intrinsic nanochannels acting as the primary mass transfer pathways within the PA matrix.”

Lines 442-445 on Page 22 in the revised manuscript.

“The cross-section of AWC membranes was investigated by TEM. Membrane coupons were first embedded in epoxy resin, and an ultramicrotome equipped with a diamond knife was then used to slice the resin-embedded membranes to prepare TEM cross-sectional samples. The resulting samples were mounted onto carbon-coated TEM grids for further characterization.”

Lines 455-457 on Page 23 in the revised manuscript.

“XRD (Bruker D8 ADVANCE, Cu K α radiation, $\lambda=1.54056$ Å) was used to characterize the structure of OH AWCs in the PA layer of RO membranes.”

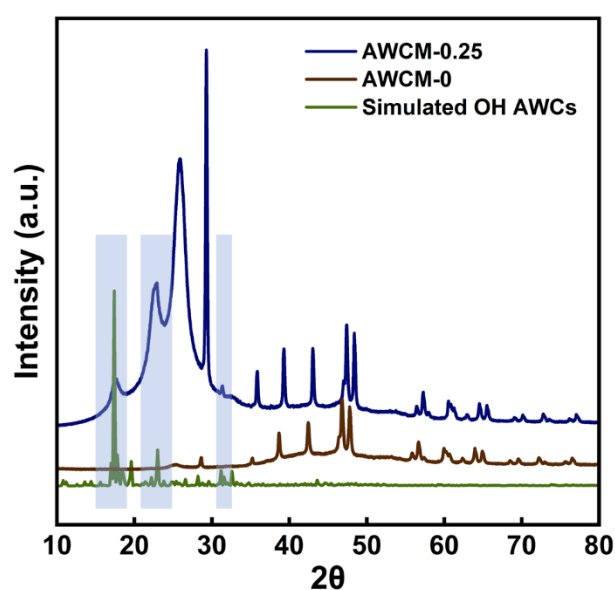


Fig. S20. Simulated XRD pattern of OH AWCs and experimentally measured XRD patterns of AWCm-0 and AWCm-0.25.

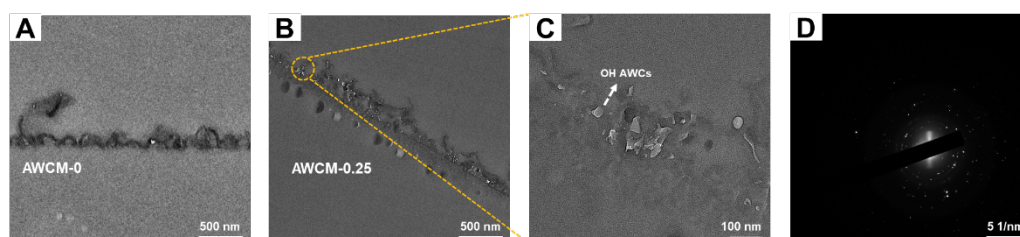


Fig. S21. Cross-sectional characterization of (A) AWCm-0 and (B) AWCm-0.25. (C) High-magnification TEM cross-section of AWCm-0.25. (D) Electron diffraction pattern of OH AWCs within the PA matrix.

(3) Several experimental results have been included to show the presence of water channels in the membranes such as WAXS, SEM, Raman, FT-IR etc. But I think those are the results that support the existence of octyl-ureido-polyol (1-(1,3-79 dihydroxypropan-2-yl)-3-octylurea molecules, not self-assembled water channels. For

example, in Fig. 2 A-C, those look just hydroxy-based molecules aggregates, not precisely and molecularly assembled water channel structures. In Fig. 2G, even though the authors argued that these data support the existence of 3.5Å pore sizes, I am not sure that amorphous diffraction peaks can be interpreted into such way the authors suggested. This result may challenge the author's interpretation because, continued from the above comments, if there were actually the self-assembled water channel structures, I would expect more clear diffraction peaks coming from the water channel structures.

Our response: Thanks for the incisive suggestion. We have confirmed through MD simulations that OUA self-assembles to form OH AWCs. Relevant descriptions have been added to the revised manuscript.

Lines 111-115 on Page 6 in the revised manuscript.

“MD simulations further confirmed that the radius of gyration of OUA decreased (the molecules assumed a more compact conformation and underwent self-assembly) in water/ethanol solution (**Supplementary Figs. S4-S6**), and that OUA self-assembled into ordered OH AWCs through H-bonds interactions (**Supplementary Figs. S7-S8**), exhibiting well-defined structural diffraction peaks (**Supplementary Fig. S9**).”

Lines 117-119 on Page 6 in the revised manuscript.

“Additionally, MD simulations confirmed that the pores of OH AWCs self-assembled from OUA were ~3.5 Å (**Supplementary Fig. S10**).”

Lines 525-526 on Page 26 in the revised manuscript.

“The simulation of OUA self-assembly into OH AWCs in water/ethanol solution was detailed in **Supplementary Text S5**.”

Details of the simulation process of OUA self-assembly into OH AWCs have been provided in the supporting information (**Text S5**) as follows:

“The self-assembly of OUA into OH AWCs in water/ethanol solution was investigated through all-atom MD simulations using the Large-scale Atomic/Molecular Massively Parallel Simulator (LAMMPS) package¹⁴. The Optimized Potentials for Liquid Simulations All-Atom (OPLS-AA) force field¹⁵ was employed to describe bonded and non-bonded interactions. Initial mixed systems were constructed and pre-optimized using energy minimization with the conjugate gradient algorithm to remove unfavorable contacts prior to dynamic sampling. Accelerated sampling techniques, including temperature annealing and bias potentials, were used to efficiently explore configurational space and promote channel formation. Equilibration and production runs were conducted in the isothermal–isobaric (NPT) ensemble at 300 K and 1 atm using the Nosé–Hoover thermostat and barostat¹⁶. The integration time step was set to 1 femtosecond (fs), and each production trajectory was propagated for 1000 picoseconds (ps) to follow the complete assembly process. The main control variable monitored throughout the

simulations was the characteristic bond length involved in water channel formation, allowing correlation between geometric changes and assembly kinetics. Periodic boundary conditions were applied in all directions, and long-range electrostatics were treated using the particle–particle particle–mesh (PPPM) Ewald method with a real-space cutoff of 12 Å. Van der Waals interactions were modeled with Lennard–Jones potentials and the same cutoff. Post-processing of MD trajectories was performed using in-house Python scripts together with the MD Analysis library¹⁷ to extract structural and dynamical descriptors of the self-assembly process. Quantities analyzed included the radius of gyration, H-bonds interactions, the pore size distribution of the formed OH AWCs, and their structural diffraction peaks. This combined approach enabled direct observation of OH AWCs formation and provided mechanistic insight into the self-assembly pathway under near-ambient conditions.”

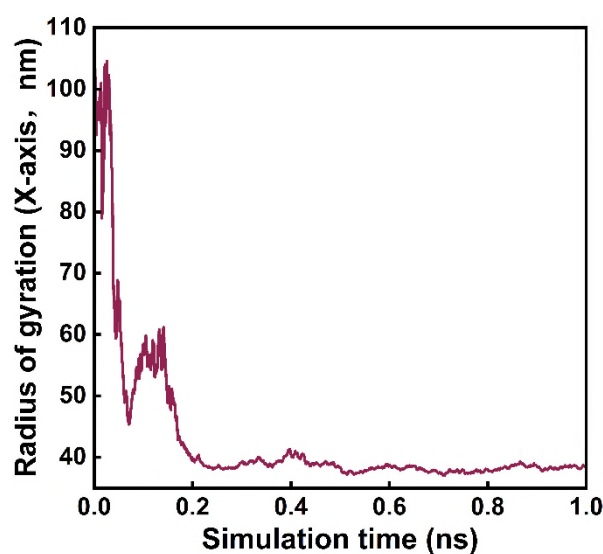


Fig. S4. Variation of the radius of gyration (X-axis) of OUA molecules in water/ethanol solution as a function of simulation time.

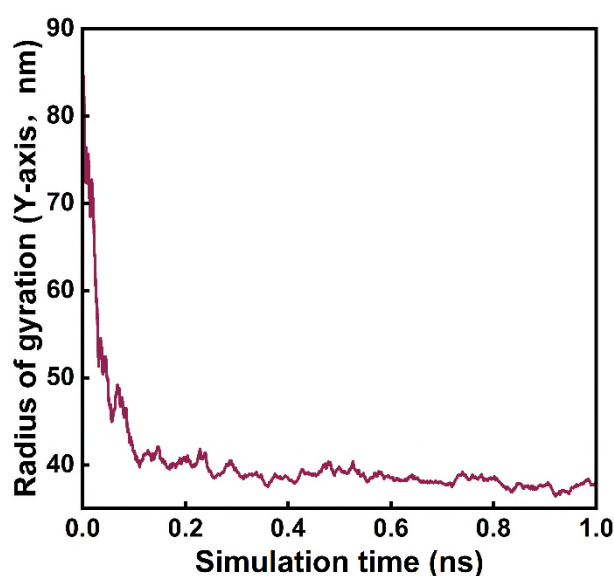


Fig. S5. Variation of the radius of gyration (Y-axis) of OUA molecules in water/ethanol solution as a function of simulation time.

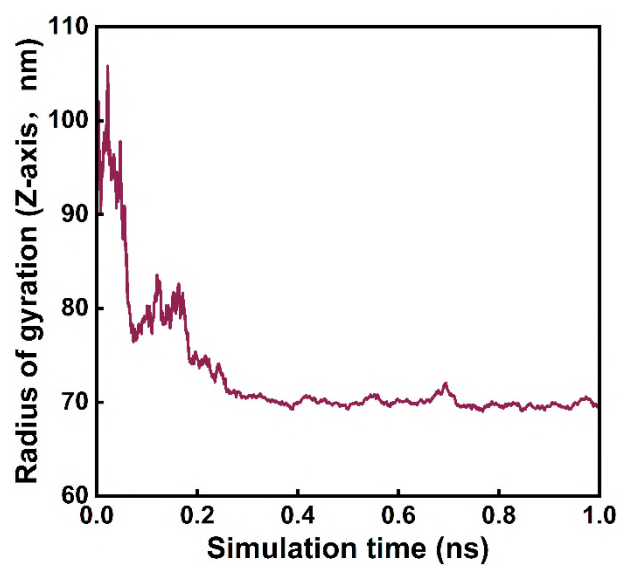


Fig. S6. Variation of the radius of gyration (Z-axis) of OUA molecules in water/ethanol solution as a function of simulation time.

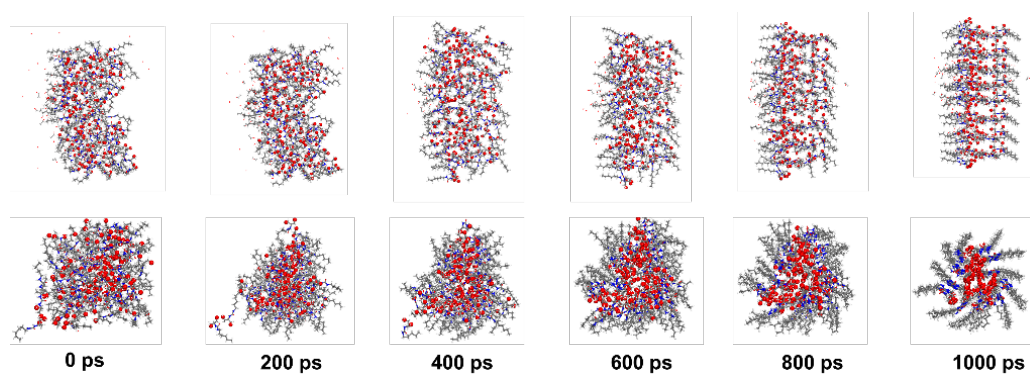


Fig. S7. The self-assembly process of OUA into OH AWCs in water/ethanol solution.

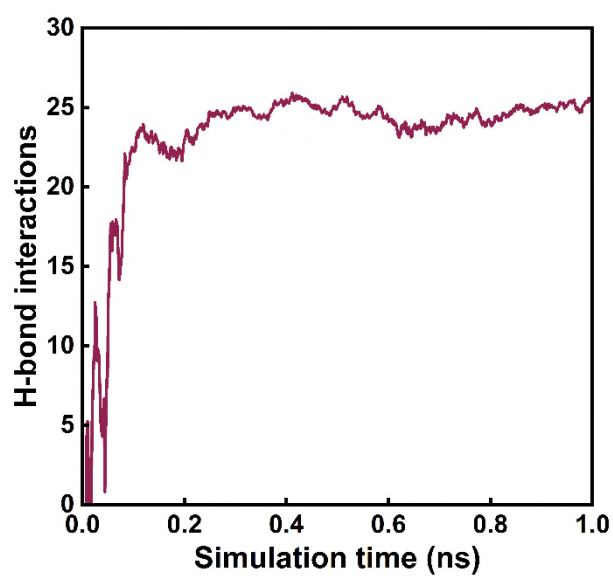


Fig. S8. H-bond interactions during the self-assembly of OUA into OH AWCs.

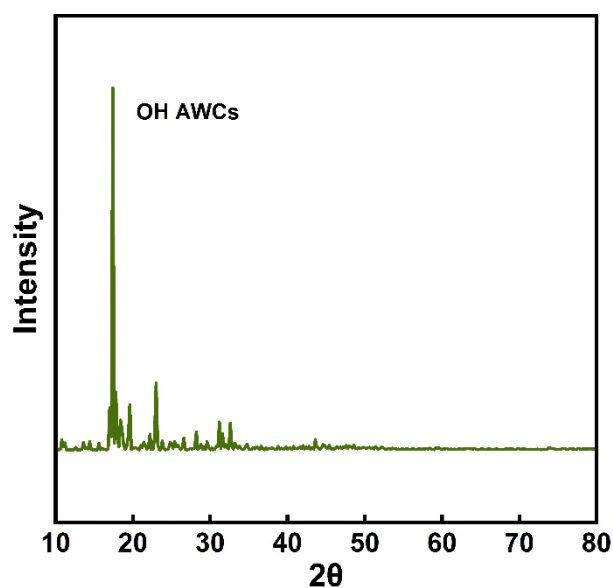


Fig. S9. Simulated XRD pattern of OH AWCs.

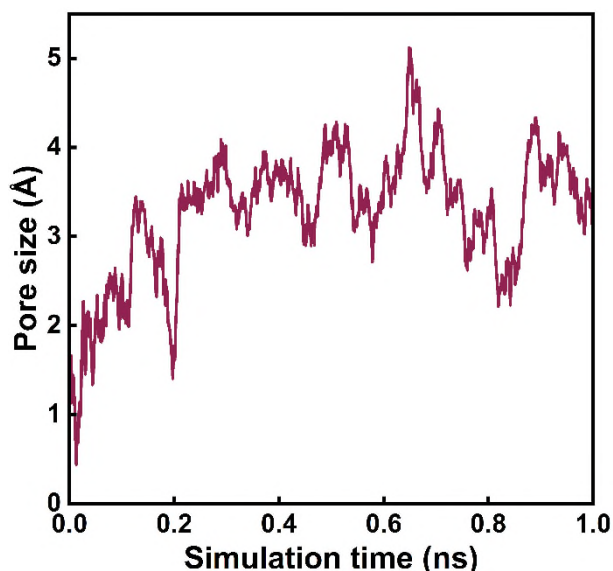


Fig. S10. Pore size distribution of OH AWCs self-assembled from OUA.

(4) In Fig. 1B, is there any experimental results to support the H-bond formation between water channel molecules and MPDs? Or is it just suggesting one possibility? I am curious whether such a well aligned structures by H-bonds between hydroxy molecules and MPDs are maintained inside PA layer or not.

Our response: Thanks for the incisive suggestion. We confirm through MD simulations that OH AWCs are incorporated into the PA layer of RO membranes via H-bonds interactions. In addition, XRD patterns of OH AWCs and AWCM membranes, along with cross-sectional TEM images of AWCM and electron diffraction patterns of OH AWCs within AWCM, demonstrate that the OH AWCs largely retained their intact structures within the PA layer. Relevant descriptions have been added to the revised manuscript.

Lines 127-131 on Page 7 in the revised manuscript.

“MD simulations further confirmed that the urea groups on the branched chains of OH AWCs interact with the -NH₂ groups of MPD via H-bonds to form MPD-OH AWCs assembled phases, which subsequently react with trimesoyl chloride (TMC) to enable the incorporation of OH AWCs into the PA layer (where OH AWCs are fixed within the PA matrix through H-bonds interactions) (**Supplementary Figs. S13-S15**).”

Lines 158-168 on Page 9 in the revised manuscript.

“In addition, X-ray diffraction (XRD) characterization was performed to analyze the structure of OH AWCs within the PA layer. Compared with the pristine RO membrane, the AWCM exhibited a red shift in the diffraction peak (**Supplementary Fig. S20**), which was attributed to the incorporation of OH AWCs into the PA layer. Moreover, characteristic diffraction peaks of OH AWCs were detected on the surface of the AWCM membrane, indicating that OH AWCs retained their intrinsic structural features within the PA layer. Cross-sectional characterization of the AWCM membranes further confirmed the successful distribution of OH AWCs within the PA matrix (**Supplementary Fig. S21(A-C)**). Moreover, OH AWCs retained well-defined lattice structures in the PA matrix (**Supplementary Fig. S21D**), indicating that they remained structurally intact and favoring their intrinsic nanochannels acting as the primary mass transfer pathways within the PA matrix.”

Lines 526-528 on Page 26 in the revised manuscript.

“MD simulations were employed to investigate the process of OH AWCs incorporation into the PA layer of RO membranes and the stability of OH AWCs under high-salinity conditions, with detailed procedures provided in **Supplementary Text S6**.”

Details of the simulation process for OH AWCs incorporation into the PA layer have been provided in the supporting information (**Text S6**) as follows:

“MD simulations were employed to investigate the process of OH AWCs incorporation into the PA layer of RO membranes, with the molecular ratio of OH AWCs:MPD:TMC:*n*-hexane:water set to 8:100:100:1965:16145. Furthermore, MD simulations were conducted to analyze the changes in H-bonds within OH AWCs in water (the number ratio of OH AWCs to water molecules was 8:7942) and high-salinity environments (the NaCl concentration was 40 g/L), in order to investigate the structural stability of OH AWCs under high-salinity conditions. The initial configurations of the solvent systems were constructed using PACKMOL¹⁸, with all molecules randomly inserted into a cubic simulation box. The OPLS-AA force field^{19, 20} was employed, consisting of bonded and nonbonded interactions, with the latter including van der Waals (vdW) and electrostatic interactions. The nonbonded interactions include vdW and electrostatic interactions, which are described by the following equations:

$$E_{LJ}(r_{ij})=4\epsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12}-\left(\frac{\sigma_{ij}}{r_{ij}}\right)^6\right) \quad (S8)$$

$$E_c(r_{ij}) = \frac{q_i q_j}{4\pi\epsilon_o\epsilon_r r_{ij}} \quad (\text{S9})$$

For different types of atoms, the Lorentz–Berthelot mixing rules were adopted for vdW interactions, as defined in Equation S10. The cutoff distance for vdW and electrostatic interactions was set to 1.2 nm, and the particle mesh Ewald (PME) method was employed to calculate long-range electrostatic interactions.

$$\sigma_{ij} = \frac{1}{2}(\sigma_{ii} + \sigma_{jj}); \epsilon_{ij} = (\epsilon_{ii} * \epsilon_{jj})^{1/2} \quad (\text{S10})$$

For this simulation, energy minimization was first performed to relax the simulation box. Then, an isothermal–isobaric (NPT) ensemble with a 1.0 fs time step was employed to optimize the simulation box, with the temperature set to 298 K and the pressure set to 1.0 atm. The temperature and pressure were maintained using the Nosé–Hoover thermostat and the Parrinello–Rahman barostat, respectively. The NPT simulation was run for 10.0 ns, which was sufficiently long to achieve a stable box size. Following the NPT simulation, a canonical (NVT) ensemble was performed for 40.0 ns to further optimize the simulation box, with the time step set to 2.0 fs. Covalent cross-linking bonds were programmatically formed between the reactive groups of TMC and MPD monomers using an in-house script that detected interatomic distances below a threshold of 5 Å and dynamically created bonds during the initial system setup phase. A canonical (NVT) ensemble was performed for 40.0 ns to further optimize the simulation box, with the time step set to 2.0 fs. In all MD simulations, the motion of atoms was described by classical Newton’s equations of motion, which were solved using the velocity-Verlet algorithm. All simulations were performed using the GROMACS 2025.1 package²¹.

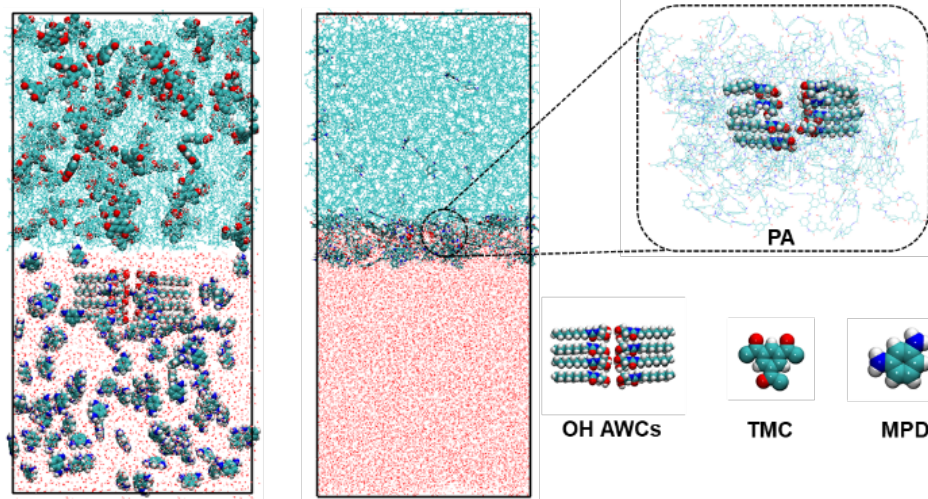


Fig. S13. MD simulation of the process by which OH AWCs are incorporated into the PA layer of RO membranes.

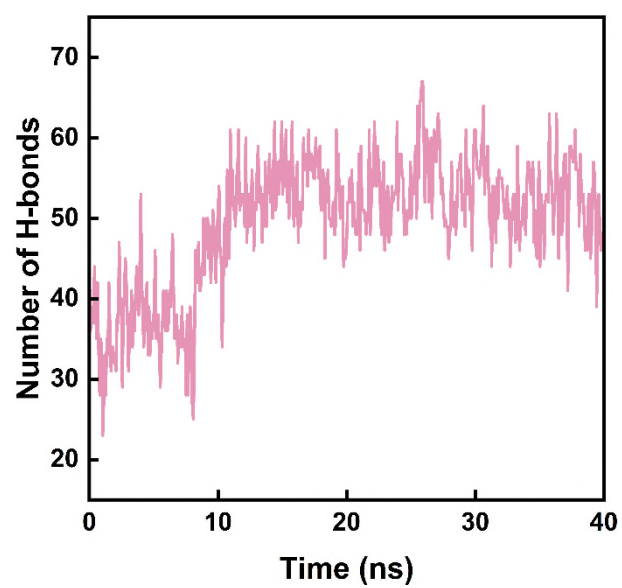


Fig. S14. H-bonds interactions between OH AWCs and MPD.

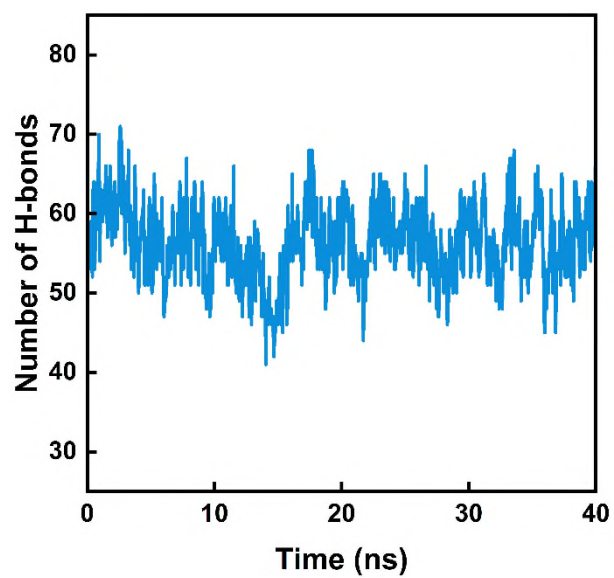


Fig. S15. H-bonds interactions between OH AWCs and PA layer.

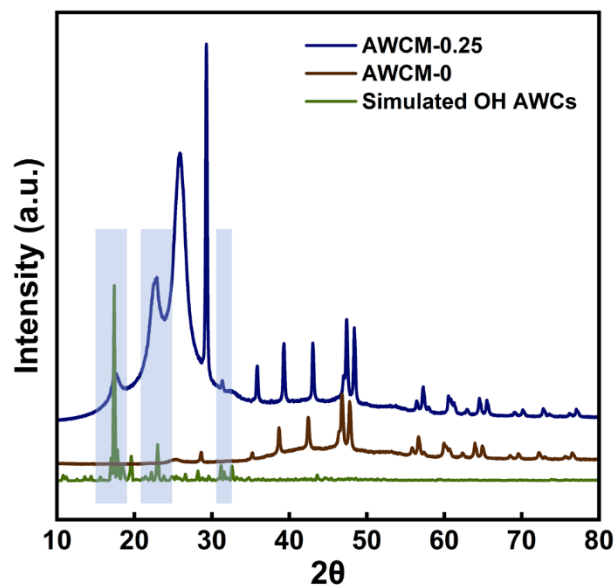


Fig. S20. Simulated XRD pattern of OH AWCs and experimentally measured XRD patterns of AWC-0 and AWC-0.25.

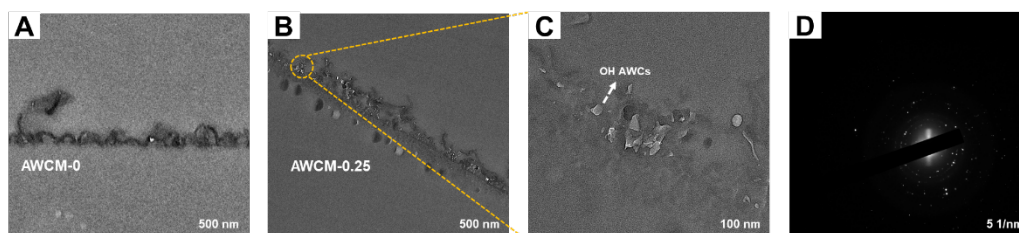


Fig. S21. Cross-sectional characterization of (A) AWC-0 and (B) AWC-0.25. (C) High-magnification TEM cross-section of AWC-0.25. (D) Electron diffraction pattern of OH AWCs within the PA matrix.

Comment #2: In the page 11, line 194, is the statement about the stability supported by any results?

Our response: Thanks for the incisive suggestion. The stability of AWC in high salinity environments is attributed to the rigidity of OH AWCs. We now elucidate the rigidity of OH AWCs in high-salinity environments by investigating the changes in H-bonds between urea groups in pure water and high-salinity environments, as well as the size variation of individual OH AWCs under high-salinity conditions. The results demonstrated that the H-bonds between urea groups in the OH AWCs structure remained highly stable under high salinity conditions, enabling the size of individual OH AWCs to remain nearly unchanged compared with pristine dimensions. This structural stability enables OH AWCs to resist the adverse effects of high salinity, thereby imparting superior rigidity to the RO membranes. Relevant descriptions have been provided in the revised manuscript.

Lines 239-247 on Pages 12-13 in the revised manuscript.

“The stability of AWCs in high salinity environments might be attributed to the rigidity of OH AWCs, which serve as the primary mass transfer channels, preventing salt ion accumulation in the PA matrix and mitigating the adverse effects of high salinity on the PA layer. The nearly unchanged average size of OH AWCs under high-salinity conditions (~22.9 nm, **Supplementary Fig. S29**) compared with their pristine dimensions (~22.7 nm, **Fig. 2E** and **Supplementary Fig. S11B**) further confirms their excellent structural rigidity. The rigidity of OH AWCs is attributed to the stability of the continuous and orderly H-bonds between urea groups, evidenced by the nearly unchanged number and lifetime of these H-bonds in pure water and high salinity environments (**Supplementary Figs. S30-S33**).”

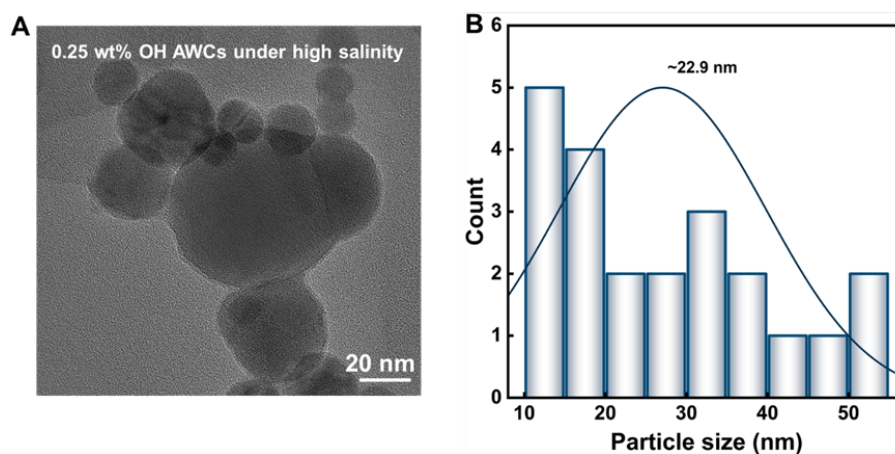


Fig. S29. (A) TEM images of 0.25 wt% OH AWCs under high-salinity conditions. (B) Size distribution of 0.25 wt% OH AWCs under high-salinity conditions, determined from TEM images using Nano Measurer 1.2 software.

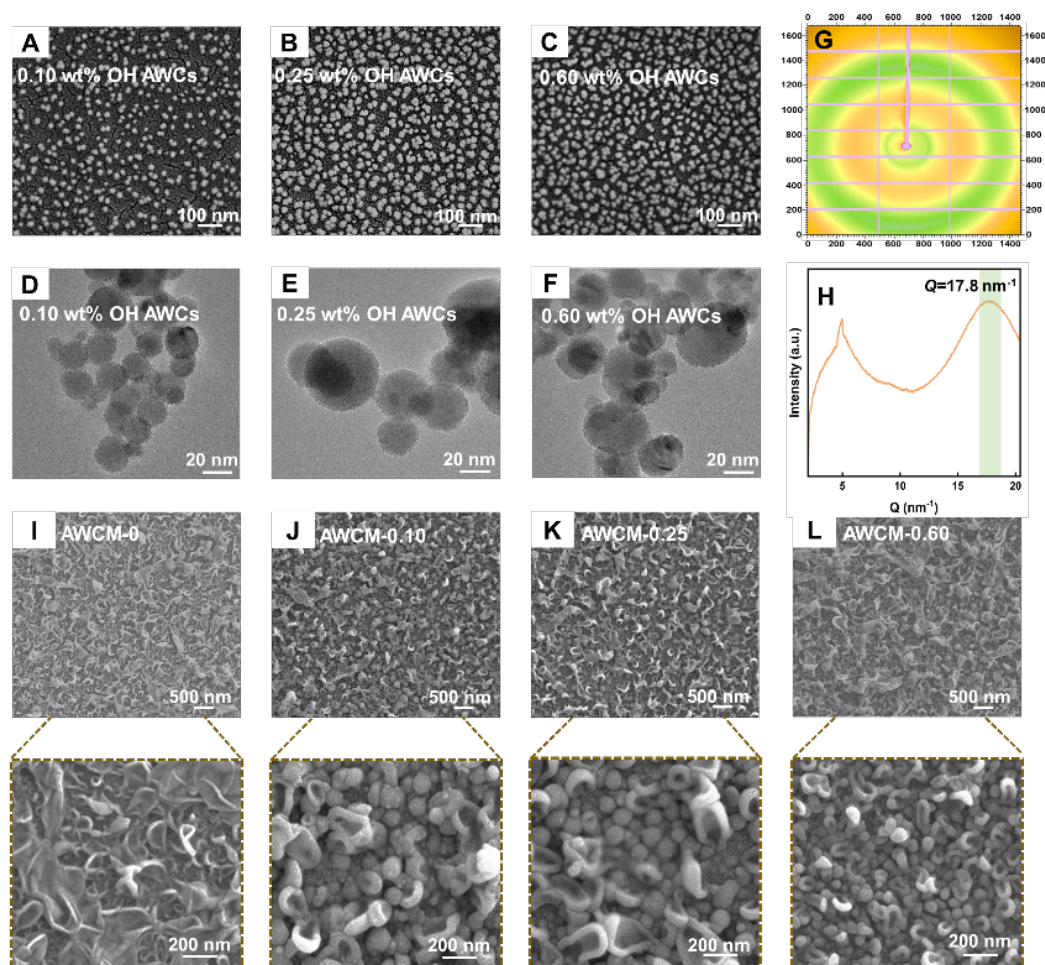


Fig. 2. Characterizations of OH AWCs and AWC membrane. (A-C) Scanning electron microscopy (SEM) images of OH AWCs. (D-F) Transmission electron microscope (TEM) images of OH AWCs. (G-H) The wide-angle X-ray scattering (WAXS) spectra of OH AWCs. The 1-(1,3-dihydroxypropan-2-yl)-3-octylurea at concentrations of 0.1 wt%, 0.25 wt%, and 0.6 wt% self-assembled into OH AWCs in an ethanol/water solution, then deposited onto the surface of polysulfone membranes and dropped onto a copper mesh for SEM and TEM characterization, respectively. (I-L) Morphology of the AWCM-0 (without OH AWCs), AWCM-0.10, AWCM-0.25, and AWCM-0.60 membranes (AWCM-0.10, AWCM-0.25, and AWCM-0.60 represented RO membranes modified with OH AWCs self-assembled from 0.1 wt%, 0.25 wt%, and 0.6 wt% 1-(1,3-dihydroxypropan-2-yl)-3-octylurea, respectively).

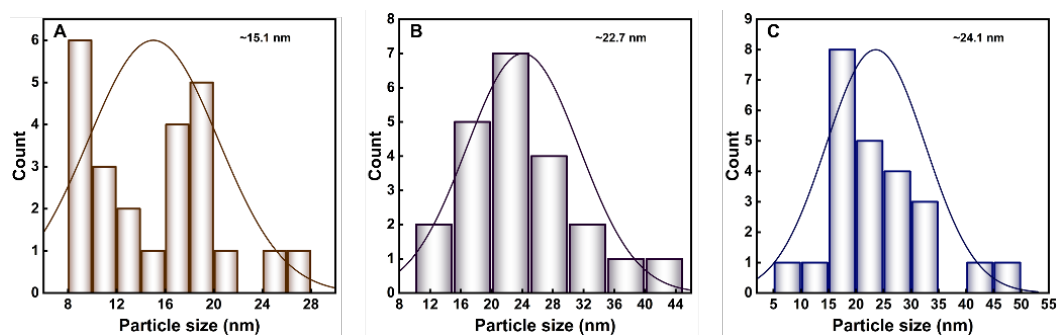


Fig. S11. Size distribution of OH AWCs at (A) 0.10 wt%, (B) 0.25 wt%, and (C) 0.60 wt%,

calculated from TEM images using Nano Measurer 1.2 software.

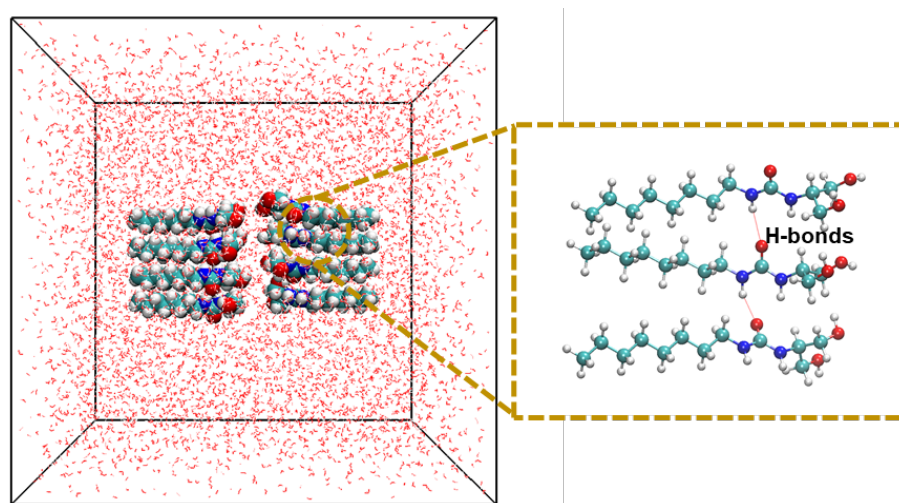


Fig. S30. Equilibrium snapshot from MD simulation of the OH AWCs in pure water.

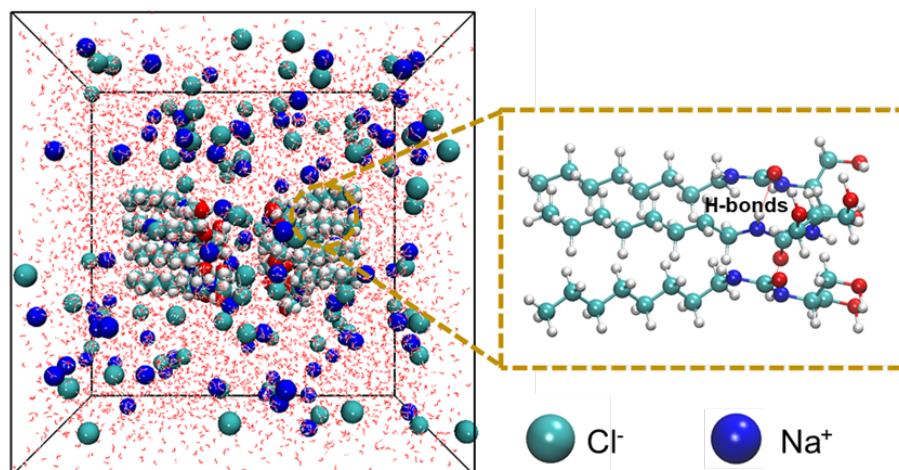


Fig. S31. Equilibrium snapshot from MD simulation of the OH AWCs in NaCl solutions (40 g/L).

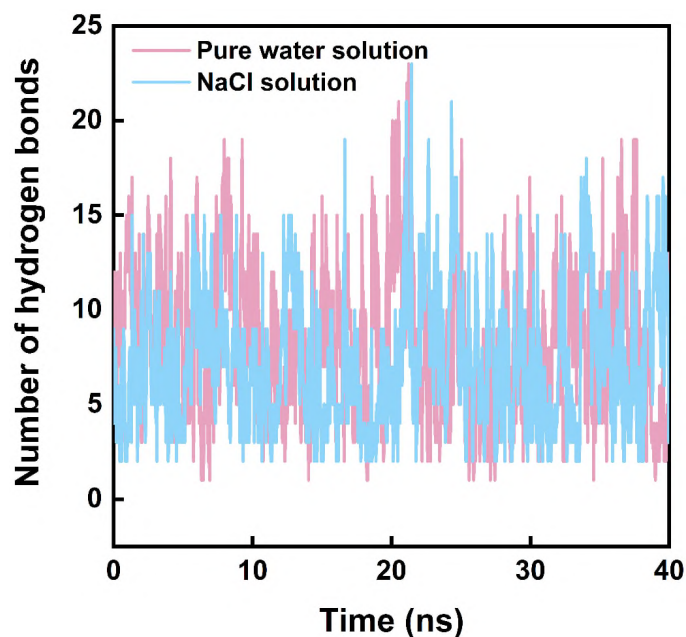


Fig. S32. Number of H-bonds between urea groups in the OH AWCs structure in pure water solution and high salinity environments (40 g/L).

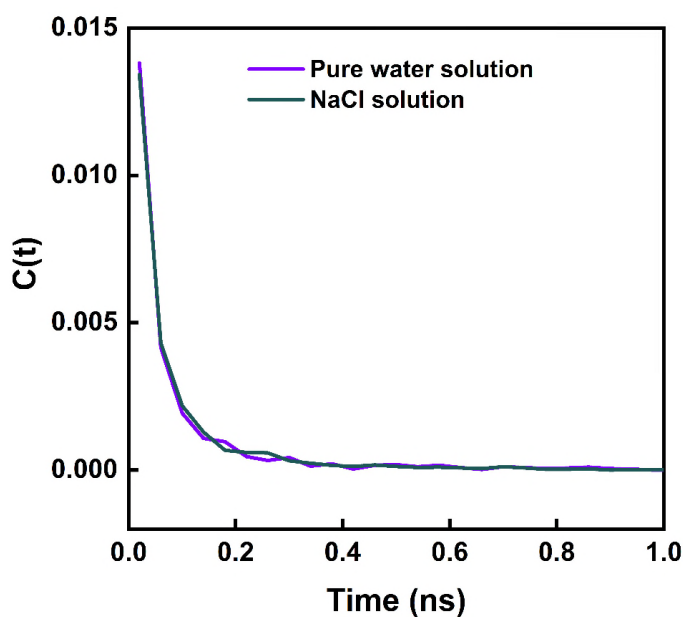


Fig. S33. Lifetime of H-bonds between urea groups in the OH AWCs structure in pure water solution and high salinity environments (40 g/L) (H-bonds population time correlation function, $C(t)$).

Comment #3: In Fig. 4, I am not sure that the GNP clusters the evidence of water permeation spots. In general, GNPs are very prone to aggregate and those particles are stabilized by some surfactants. Hydroxy water channel molecules are amphiphilic and can serve as surfactant as well, probably just replacing the original surfactant and induce GNP aggregation or cluster formation near the spot where hydroxy water channels exist.

Our response: Thanks for the incisive suggestion. In our study, citrate-stabilized GNPs were used. Citrate reduces AuCl_4^- to form GNPs, and the $-\text{COO}^-$ groups of citrate bind to the GNP surface via inner-sphere complexation, rendering the GNPs negatively charged and generating electrostatic repulsion that suppresses aggregation, thereby producing a stable and well-dispersed colloid (*Journal of the American Chemical Society*, 2014, 136: 1907-1921; *Journal of Colloid and Interface Science*, 1994, 165: 97-109). Therefore, even though OH AWCs are amphiphilic, they are unlikely to replace the stabilizing role of citrate. Moreover, visualizing water permeation sites through GNPs filtration experiments is an effective strategy (*Science*, 2018, 360: 518-521). Under neutral conditions, GNPs are negatively charged and exhibit surface charge behavior similar to that of RO membranes. For GNPs deposition to occur, the drag force must overcome the electrostatic repulsion between the GNPs and the membrane surface, and therefore the resulting spatial distribution of GNPs is primarily governed by the water transport pathways. Consequently, the deposition sites of GNPs correspond to the water permeation sites, providing direct visual evidence of water transport pathways. Relevant descriptions have been provided in the revised manuscript.

Lines 251-255 on Page 13 in the revised manuscript.

“Under neutral conditions, GNPs are negatively charged and exhibit similar surface charge behavior to RO membranes (**Supplementary Fig. S34**)⁴¹. For GNPs deposition to occur, the drag force must overcome the electrostatic repulsion between the GNPs and the membrane surface, and therefore the resulting spatial distribution of GNPs is primarily governed by the water transport pathways⁴¹.”

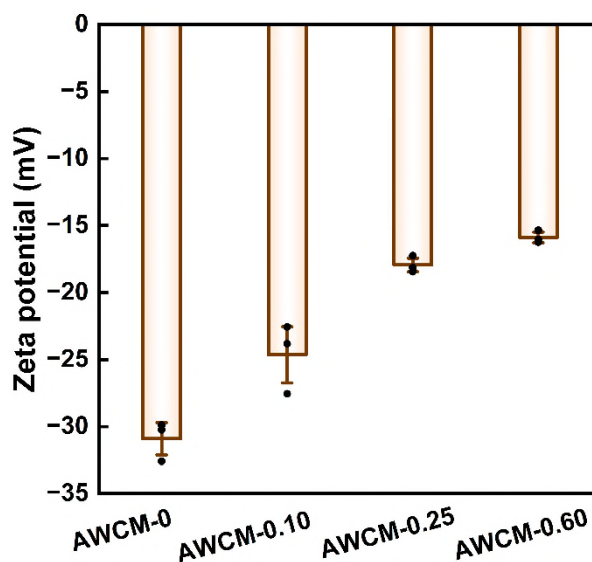


Fig. S34. The surface charge of AWC0, AWC0.10, AWC0.25, and AWC0.60 membranes. Error bars represent the s.d. ($n = 3$) and data are presented as mean values $\pm$ s.d.

Comment #4: In the page 19, line 344, the authors claimed that this manuscript is the first to elucidate the mechanism by which AWCs enhance the performance of RO

membranes. I would suggest reviewing some references I added in the first paragraph of this comment file.

Our response: Thanks for the incisive suggestion. We have carefully reviewed the references provided by the reviewer. Although the above literature reported that incorporating AWCs into the PA layer of RO membranes can enhance membrane separation performance, they did not elucidate how AWCs participate in and govern the mass transfer process within the mixed rejection layer of AWCs and PA. Our study demonstrates that AWCs serve as the primary mass transfer channels in the mixed rejection layer of AWCs and PA, thereby determining the separation performance of the RO membrane and elucidating the distinct mass transfer mechanisms of water molecules and solutes within the AWCs. Relevant descriptions have been provided in the revised manuscript.

Lines 72-75 on Page 4 in the revised manuscript.

“Nevertheless, the mysterious role of AWCs when coexisting with the PA rejection layer, whose nanochannels may play a critical role in mass transfer and largely determine the overall membrane performance, remains unexplored.”

Lines 76-81 on Page 4 in the revised manuscript.

“Consequently, AWCs could emerge as the primary mass transfer channels in RO membranes, dictating the overall separation performance. The potential role of AWCs as the primary mass transfer channels is elucidated for the first time, which could facilitate water molecule transport while inhibiting solute diffusion, thereby breaking through the upper bound of RO membrane performance and achieving simultaneous enhancement of water permeance and NDMA rejection.”

Comment #5: In MD simulation part, how the authors constructed the water channel structure model? For performing MD simulations, the most important and challenging part may how to construct molecular structures that represent the real chemical structure of the water channels or membrane matrix. I tried to find out the details how the authors constructed these OH AWCs, the PA matrix, and the AWCMs, but couldn't find out. Please clarify it and suggest that these structure are well representing the actual microstructures of the OH AWCs, PA matrix, and AWCMs.

Our response: Thanks for the incisive suggestion. We have now provided detailed descriptions of the modeling procedures in the supporting information to clarify this point. The relevant description has been added to the supporting information as **Text S8**.

“The structural models of the OH AWCs, PA matrix, and AWCM were constructed through a hierarchical modeling protocol designed to closely reproduce their experimentally reported microstructures. The OH AWCs were first built according to their chemical formulas and three-dimensional structures reported in synthetic studies¹⁰, and the initial coordinates were

generated in Avogadro and optimized by density functional theory calculations at the B3LYP/6-31G(d) level. Electrostatic potential-derived RESP charges were obtained from HF/6-31G(d) calculations, and the GAFF2 force field was employed to describe bonded and nonbonded interactions, with key torsional parameters refined against quantum chemical scans. A crosslinked PA network was generated via interfacial polymerization of MPD and TMC using the Polymatic algorithm, yielding structural parameters consistent with experimental data. After polymerization, the network was equilibrated at 300 K and 1 atm to relax the free volume and pore structure, yielding structural features in close agreement with experimental measurements of PA membranes. AWCM models were constructed by embedding multiple optimized OH AWCs into the equilibrated PA matrix using the Packmol algorithm. The composite systems were subsequently solvated with explicit TIP4P-Ew water molecules, neutralized with counterions, and equilibrated in a stepwise manner, beginning with restrained solvent relaxation and followed by extended NPT equilibration to allow relaxation of both the PA matrix and channel structures. Structural stability was confirmed by monitoring pore diameters, radial distribution functions, and free volume distributions, all of which indicated preservation of the OH AWCs geometry and realistic packing of the PA matrix. This modeling workflow ensured that the constructed OH AWCs, PA network, and AWCM closely reproduced the key microstructural features observed experimentally.”

Reviewer #3:

The authors polyamide RO membranes embedded with a previously reported artificial water channels with aligned hydroxyl groups to address a critical limitation in RO membranes: the poor rejection of small, uncharged, and carcinogenic molecules such as NDMA. Incorporating OH AWCs into the PA layer leads to over 30% improvement in NDMA rejection compared to the unmodified PA membrane, while maintaining high water permeance. The resulting membrane surpasses the conventional selectivity–permeability trade-off observed in RO membranes, potentially reducing the need for downstream treatment processes to remove NDMA. The role of AWCs is supported by a combination of experimental characterization and molecular dynamics (MD) simulations. This study addresses an important challenge in water reuse and proposes a promising design strategy. However, several critical concerns remain before I can recommend for publication. These issues should be addressed to strengthen the validity of the core claims.

Our response: Thanks for dedicating your time and providing a thoughtful review. In response to your incisive suggestions, we have carefully incorporated them into the revised manuscript. Detailed point-by-point responses are provided below.

1. The central claim is that OH AWCs are responsible for the enhanced NDMA rejection. While the authors present control membranes with SiO₂ to inhibit AWC activity and observe reductions in water permeance and NaCl rejection, they do not report NDMA rejection for these inhibited membranes. Since NDMA rejection is the focal point of the study, it is essential to demonstrate that removing AWC functionality also compromises NDMA exclusion.

Our response: Thanks for the incisive suggestion. We have added NDMA rejection data for RO membranes incorporated with SiO₂@OUA (aggregates formed by the self-assembly of SiO₂ and OUA). The results showed that NDMA rejection decreased significantly in SiO₂@OUA modified RO membranes. Relevant descriptions have been added to the revised manuscript.

Lines 291-293 on Page 15 in the revised manuscript.

“The salt rejection of the AWCM-0.25I decreased from $99.2 \pm 0.3\%$ to $97.9 \pm 0.4\%$ (Fig. 5C), and the NDMA rejection decreased from $93.9 \pm 0.1\%$ to $65.5 \pm 0.8\%$ (Supplementary Fig. S44).”

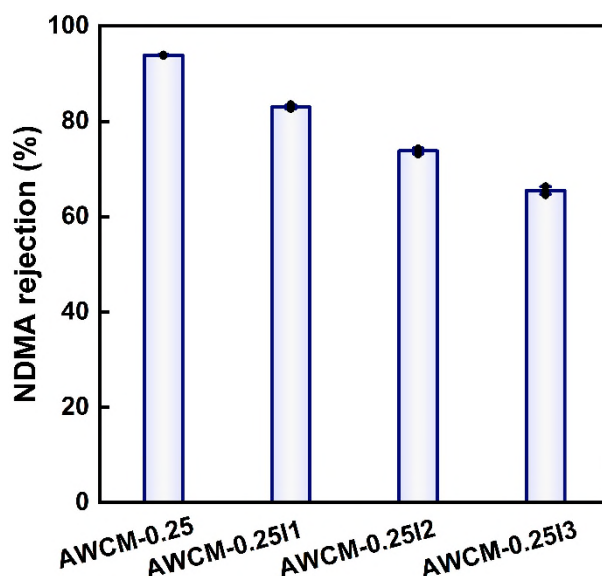


Fig. S44. The NDMA rejection of the AWCm-0.25, AWCm-0.25I1, AWCm-0.25I2, and AWCm-0.25I3. Error bars represent the s.d. ($n = 3$), and data are presented as mean $\pm$ s.d.

2. The inhibitory control membranes contain both SiO_2 and OUA. However, it is unclear whether SiO_2 alone could impact PA morphology or transport properties. The absence of a control membrane prepared with SiO_2 alone makes it difficult to isolate the specific role of SiO_2 as an inhibitor. Including this control would clarify whether the observed changes in transport are due to inhibited AWC formation or other effects from the SiO_2 additive.

Our response: Thanks for the incisive suggestion. We have now added the morphology, water permeance, and NaCl and NDMA rejection data of RO membranes modified solely with SiO_2 to confirm that the performance decline of SiO_2 @OUA modified RO membranes was caused by the inhibition of OH AWCs formation. Relevant descriptions have been provided in the revised manuscript.

Lines 293-298 on Page 15 in the revised manuscript.

“Furthermore, compared with the AWCm-0 membrane, the surface morphology, pure water permeance, and NaCl and NDMA rejection of RO membranes modified solely with SiO_2 showed no significant changes (Supplementary Figs. S45-S48), indicating that the decline in the separation performance of the AWCm-0.25I membrane was primarily attributed to the reduced quantity of available OH AWCs for water molecule and NaCl transport within the membrane, which shifted the primary transport pathways toward the PA matrix.”

Lines 496-499 on Page 25 in the revised manuscript.

“In addition, the amounts of SiO_2 used to inhibit OUA self-assembly were also employed for RO membrane modification and were designated as SiO_2 -RO1, SiO_2 -RO2, and SiO_2 -RO3. Subsequently, the role of OH AWCs in the mass transfer process was explored by analyzing

the surface characteristics, water permeance, and NaCl and NDMA rejection of these membranes.”

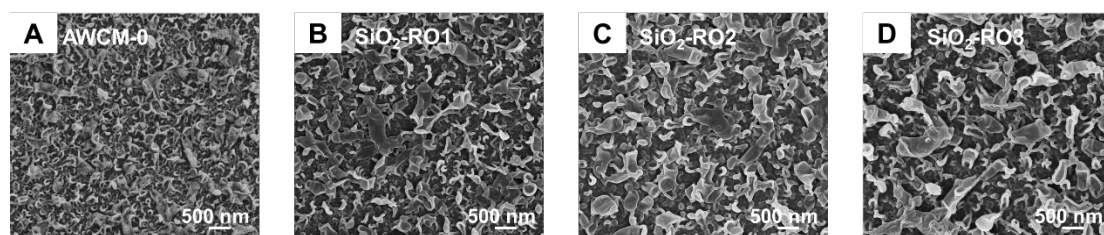


Fig. S45. SEM images of AWCM-0 and SiO₂ modified RO membranes.

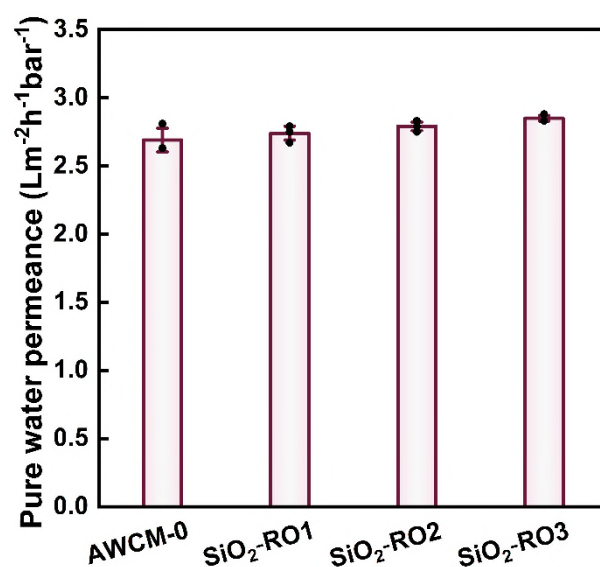


Fig. S46. Pure water permeance of AWCM-0 and SiO₂ modified RO membranes. Error bars represent the s.d. ($n = 3$), and data are presented as mean $\pm$ s.d.

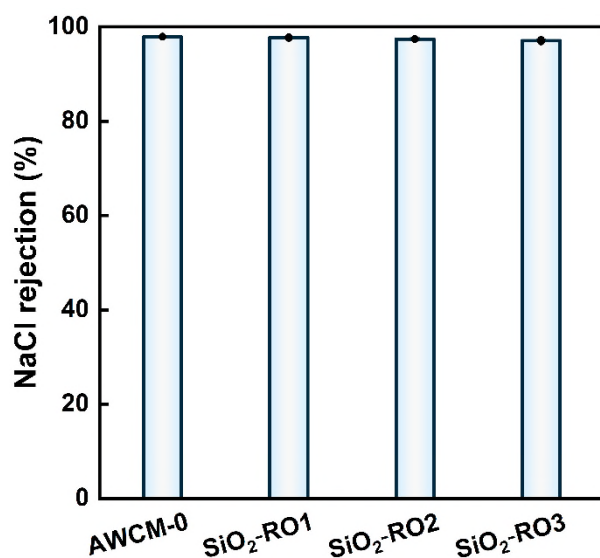


Fig. S47. NaCl rejection of AWCM-0 and SiO₂ modified RO membranes. Error bars represent the s.d. ($n = 3$), and data are presented as mean $\pm$ s.d.

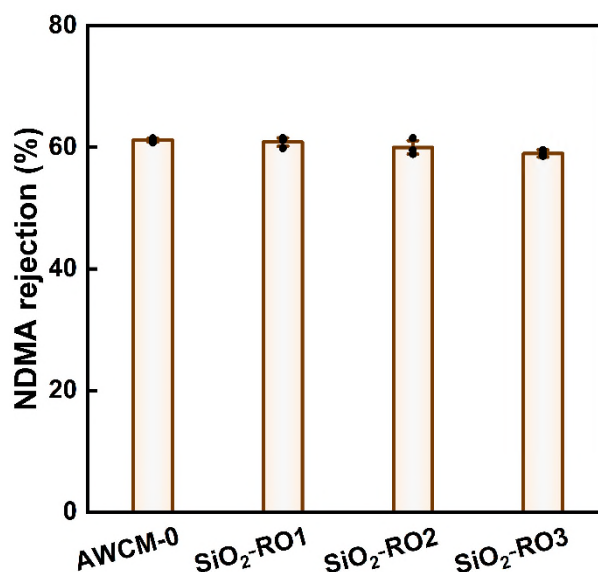


Fig. S48. NDMA rejection of AWCm-0 and SiO₂ modified RO membranes. Error bars represent the s.d. ($n = 3$), and data are presented as mean $\pm$ s.d.

3. The WAXS data demonstrating subnanometer pore formation were apparently collected from self-assembled OH AWC aggregates in isolation, not from the final membranes. Given that the AWCs are integrated during interfacial polymerization—a process involving organic solvents and chemical crosslinking—the assumption that OH AWCs retain their channel structure within the PA matrix requires experimental validation. The authors should discuss whether such structural integrity can realistically be retained and provide supporting evidence.

Our response: Thanks for the incisive suggestion. We provide XRD patterns of OH AWCs and AWCm membranes, along with cross-sectional TEM images of AWCm and the electron diffraction pattern of OH AWCs within AWCm, demonstrating that the OH AWCs largely retained their intact structures within the PA layer. Relevant descriptions have been provided in the revised manuscript.

Lines 158-168 on Page 9 in the revised manuscript.

“In addition, X-ray diffraction (XRD) characterization was performed to analyze the structure of OH AWCs within the PA layer. Compared with the pristine RO membrane, the AWCm exhibited a red shift in the diffraction peak (**Supplementary Fig. S20**), which was attributed to the incorporation of OH AWCs into the PA layer. Moreover, characteristic diffraction peaks of OH AWCs were detected on the surface of the AWCm membrane, indicating that OH AWCs retained their intrinsic structural features within the PA layer. Cross-sectional characterization of the AWCm membranes further confirmed the successful distribution of OH AWCs within the PA matrix (**Supplementary Fig. S21(A–C)**). Moreover, OH AWCs retained well-defined lattice structures in the PA matrix (**Supplementary Fig. S21D**), indicating that they remained structurally intact and favoring their intrinsic nanochannels acting as the primary mass transfer pathways within the PA matrix.”

Lines 442-445 on Page 22 in the revised manuscript.

“The cross-section of AWC membranes was investigated by TEM. Membrane coupons were first embedded in epoxy resin, and an ultramicrotome equipped with a diamond knife was then used to slice the resin-embedded membranes to prepare TEM cross-sectional samples. The resulting samples were mounted onto carbon-coated TEM grids for further characterization.”

Lines 455-457 on Page 23 in the revised manuscript.

“XRD (Bruker D8 ADVANCE, Cu K α radiation, $\lambda=1.54056$ Å) was used to characterize the structure of OH AWCs in the PA layer of RO membranes.”

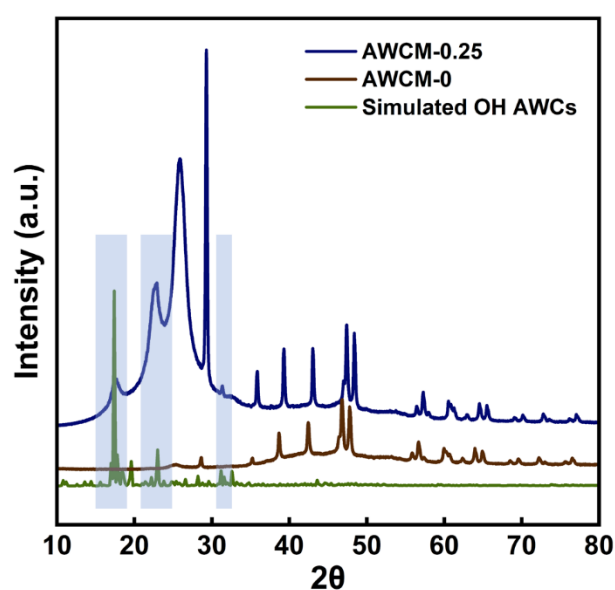


Fig. S20. Simulated XRD pattern of OH AWCs and experimentally measured XRD patterns of AWC-0 and AWC-0.25.

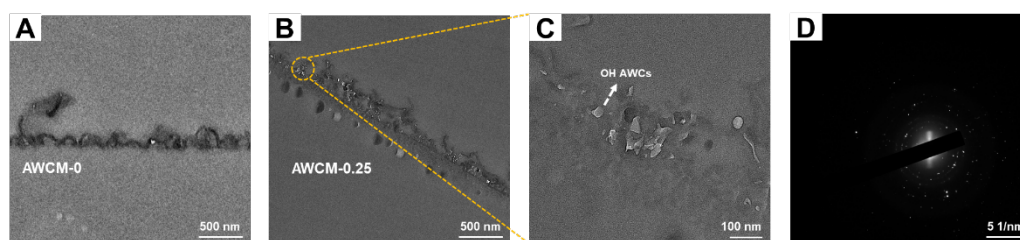


Fig. S21. Cross-sectional characterization of (A) AWC-0 and (B) AWC-0.25. (C) High-magnification TEM cross-section of AWC-0.25. (D) Electron diffraction pattern of OH AWCs in the PA matrix.

4. The authors interpret the O/N ratio from XPS as evidence of successful AWC incorporation. However, if this ratio is taken as quantitative, one would expect a monotonic increase in O/N ratio with higher AWC loading. The authors should clarify.

Our response: Thanks for the suggestion. We agree with your explanation. The O/N ratio of OH AWCs is indeed much higher than that of the conventional PA. However, the O/N ratio of the AWCM did not increase monotonically with higher OH AWCs loading, possibly because the increased cross-linking degree of the PA (which leads to a decrease in the O/N ratio) offset the enhancement in O/N ratio resulting from the incorporation of OH AWCs.

Lines 156-158 on Pages 8-9 in the revised manuscript.

“The O/N ratio of the AWCM decreased with increasing OH AWCs content, possibly because the increased cross-linking degree of the PA (which leads to a decrease in the O/N ratio) offset the enhancement in O/N ratio resulting from the incorporation of OH AWCs (**Supplementary Fig. S19**).”

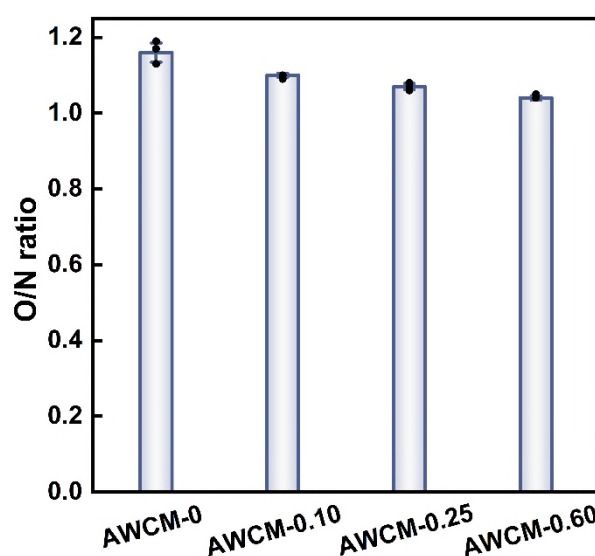


Fig. S19. The O/N ratio of AWCM-0, AWCM-0.10, AWCM-0.25, and AWCM-0.60 membranes. Error bars represent the s.d. ($n = 3$), and data are presented as mean values $\pm$ s.d.

Our response: Thanks for the incisive suggestion. We have now substantially revised our manuscript and are pleased to resubmit it to Nature Communications. Specifically, in response to Reviewer #4's suggestion, we have refined the description of the primary mass transfer channels, now defining them as OH AWCs mediated transport pathways. To address Reviewer #2's concern regarding the lack of direct evidence, we employed the infrared mapping approach suggested by Reviewer #4 to trace the spatial distribution of D₂O on the membrane surface. By providing co-localized mapping of the PA matrix, OH AWCs, and D₂O at the same micro-location, we offer direct evidence confirming that transport within the AWCM is indeed mediated by OH AWCs. Furthermore, by benchmarking the performance and transport selectivity (water vs. NDMA) of OH AWCs against I-quartet (HC6) and imidazolethyl-ureidoethyl-phenyl (IUP)-based channels, we further highlight the distinct advantages and novelty of our work. More detailed responses to the reviewer comments are provided below.

Response to Reviewer Comments and List of Changes Made in the Manuscript

Reviewer #2:

I appreciate the authors' efforts in revising the manuscript and preparing a detailed point-by-point rebuttal. The revised version is more polished in language and better organized than the original submission. The added experiments on fouling, high-salinity stability, and the SiO₂@OUA (channel inhibition system) certainly enrich the dataset. However, the core scientific concerns I had in the first round review process remain unresolved. First of all, the central mechanism that hydroxyl water channels (OH AWCs) act as the primary mass transfer channels within the polyamide (PA) layer is still not convincingly demonstrated and relies vastly on indirect evidence. Also, the novelty of the work is still limited relative to the rapidly growing literature on AWC-PA composite membranes and NDMA rejection. Several additional claims such as "excellent antifouling performance" and "first to elucidate the mechanisms by which AWCs enhance RO performance", remain overstated relative to the presented evidence. Because these points concern the main claims of the paper, I do not think they can be addressed with further revision process. Several detailed comments are as follows.

Our response: Thanks for dedicating your time and providing a thoughtful review. We have carefully revised our manuscript based on the suggestions. Point-by-point responses are provided below.

(i) However, the core scientific concerns I had in the first round review process remain unresolved. First of all, the central mechanism that hydroxyl water channels (OH AWCs) act as the primary mass transfer channels within the polyamide (PA) layer is still not convincingly demonstrated and relies vastly on indirect evidence.

Reply to point (i): We agree with your comment. Inspired by Reviewer #4, the description of the primary mass transfer channels in the manuscript has now been revised to describe them as **OH AWCs mediated transport pathways**. Meanwhile, we employed the infrared mapping approach suggested by Reviewer #4 to trace the spatial distribution of D₂O on the membrane surface. By providing co-localized mapping of the PA matrix, OH AWCs, and D₂O at the same micro-location, we offer direct evidence confirming that transport within the AWCM is indeed mediated by OH AWCs. More detailed responses are provided below.

(ii) Also, the novelty of the work is still limited relative to the rapidly growing literature on AWC-PA composite membranes and NDMA rejection.

Reply to point (ii): We understand the concern that some studies have explored the incorporation of AWCs to enhance the separation performance of RO membranes. To clarify the novelty of our study, we have provided comprehensive comparisons, highlighting the key differences between the representative studies cited by the reviewer and our work, as well as the distinct transport mechanism

imparted by OH AWCs to RO membranes compared with the HC6 AWCs and IUP AWCs previously reported for RO membrane modification. For more detailed information, please see our response to Comment 4.

(iii) Several additional claims such as “excellent antifouling performance” and “first to elucidate the mechanisms by which AWCs enhance RO performance”, remain overstated relative to the presented evidence.

Reply to point (iii): Thanks for the suggestion. We have modified “excellent antifouling performance” to “enhanced resistance to humic acid (HA) fouling” and replaced “first to elucidate the mechanisms by which AWCs enhance RO performance” with “the study elucidates the mechanisms by which AWCs mediate transport pathways and channel inner-wall functional groups induce H-bonds interactions to enhance the performance of RO membranes” in the revised manuscript. Relevant descriptions have been provided in the revised manuscript.

Lines 194-197 on Page 10 in the revised manuscript.

“Moreover, AWCM-0.25 exhibited enhanced resistance to humic acid (HA) fouling compared with AWCM-0 (**Supplementary Fig. S19**), indicating that the incorporation of OH AWCs into RO membranes not only enhanced the rejection of neutral small-molecule pollutants but also improved antifouling performance.”

Lines 385-388 on Page 20 in the revised manuscript.

“The study elucidates the mechanisms by which AWCs mediate transport pathways and channel inner-wall functional groups induce H-bonds interactions to enhance the performance of RO membranes, providing crucial guidance for the development of high-performance AWCs-based RO membranes.”

1. Evidence that OH AWCs are the “primary mass transfer channels” is still not compelling. The revised manuscript repeatedly and very strongly states that OH AWCs “serve as the primary mass transfer channels” and “govern the overall performance” of the membranes throughout the whole manuscript. While the authors have added more data such as GNP deposition patterns, Raman mapping of water distribution, SiO₂@OUA channel inhibition, and MD simulations, each suggested evidence is still indirect and can also be explained by more conventional changes in PA morphologies and free volume. For example, even though the authors claims that GNP clustering occurs near OH-AWCs, but there is no direct experimental evidence demonstrating spatial co-localization between OH-AWC domains and GNP deposition. All presented data (GNP patterns, flux differences, PA morphology changes) are indirect and can be explained by alternative factors such as local PA thickness, roughness, charge distribution, or free volume variation. Without direct co-mapping of AWC distribution and GNP deposition, the claim that GNP clusters uniquely mark the presence of AWCs is unsupported. Also, the Raman mapping does not provide any direct evidence of the spatial distribution of OH-AWCs as well. The measured OH-stretching signal merely

reflects local water content and cannot be uniquely attributed to AWC domains. Considering the spatial resolution (>500 nm) relative to the AWC particle size (~15–24 nm), the Raman hotspots cannot represent AWC locations. Thus, the assertion that Raman data undoubtedly indicate AWC-rich regions is not convincing to me.

Our response: Thanks for the incisive suggestion. We would like to reply to it in a point-by-point manner.

(i) The revised manuscript repeatedly and very strongly states that OH AWCs “serve as the primary mass transfer channels” and “govern the overall performance” of the membranes throughout the whole manuscript.

Reply to point (i): We agree with your comment. We have changed “serve as the primary mass transfer channels” to “OH AWCs mediate the transport pathways” and removed the description related to “govern the overall performance” in the revised manuscript. Relevant descriptions have been provided in the revised manuscript.

Lines 25-29 on Page 2 in the revised manuscript.

“OH AWCs mediated the transport pathways, enabling the membrane to deliver outstanding desalination performance ($99.2 \pm 0.3\%$), an exceptional NDMA rejection ($93.9 \pm 0.1\%$) that surpasses the existing upper bound of permselectivity, and an ultrahigh pure water permeance ($4.5 \pm 0.2 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1} \cdot \text{bar}^{-1}$) that exceeds that of state-of-the-art RO membranes.”

Lines 69-73 on Page 4 in the revised manuscript.

“For example, incorporating AWCs (I-quartet AWCs and imidazolylethyl-ureidoethyl-phenyl AWCs) into the PA layer improved the separation performance of the RO membranes^{30, 31}. Nevertheless, the role of AWCs when coexisting with the PA rejection layer, whether AWCs mediate the transport pathways, and how channel inner-wall functional groups influence mass transfer remain insufficiently explored.”

Lines 86-89 on Page 5 in the revised manuscript.

“Visualizing water molecule clustering regions, regulating the PA pores, and conducting molecular dynamics (MD) simulations revealed that OH AWCs mediate the transport pathways (**Fig. 1C** and **Fig. 1D**) and that channel inner-wall functional groups contribute to the enhanced performance of the AWCM.”

Lines 234-236 on Page 12 in the revised manuscript.

“The results potentially indicated that OH AWCs mediate the transport pathways, mitigating the adverse effects of the increased cross-linking degree of the PA layer on water molecule transport.”

Lines 312-316 on Page 16 in the revised manuscript.

“For NDMA, during transport through the OH AWCs, a greater number of H-bonds were formed than in the PA matrix (**Supplementary Fig. S37**), and these H-bonds exhibited longer lifetimes (**Supplementary Fig. S38**), leading to prolonged residence times and lower jumping frequencies (**Supplementary Figs. S39-S40**), consequently OH AWCs mediated transport pathways conferred a higher permeation energy barrier for NDMA (**Fig. 6I**) and superior rejection in the AWCm.”

Lines 375-376 on Page 19 in the revised manuscript.

“OH AWCs retain their intact structures within the PA matrix and mediate the transport pathways, thereby enhancing the performance of RO membranes.”

Lines 385-388 on Page 20 in the revised manuscript.

“The study elucidates the mechanisms by which AWCs mediate transport pathways and channel inner-wall functional groups induce H-bonds interactions to enhance the performance of RO membranes, providing crucial guidance for the development of high-performance AWCs-based RO membranes.”

(ii) While the authors have added more data such as GNP deposition patterns, Raman mapping of water distribution, SiO₂@OUA channel inhibition, and MD simulations, each suggested evidence is still indirect and can also be explained by more conventional changes in PA morphologies and free volume. For example, even though the authors claims that GNP clustering occurs near OH-AWCs, but there is no direct experimental evidence demonstrating spatial co-localization between OH-AWC domains and GNP deposition. All presented data (GNP patterns, flux differences, PA morphology changes) are indirect and can be explained by alternative factors such as local PA thickness, roughness, charge distribution, or free volume variation. Without direct co-mapping of AWC distribution and GNP deposition, the claim that GNP clusters uniquely mark the presence of AWCs is unsupported. Also, the Raman mapping does not provide any direct evidence of the spatial distribution of OH-AWCs as well. The measured OH-stretching signal merely reflects local water content and cannot be uniquely attributed to AWC domains. Considering the spatial resolution (>500 nm) relative to the AWC particle size (~15–24 nm), the Raman hotspots cannot represent AWC locations. Thus, the assertion that Raman data undoubtedly indicate AWC-rich regions is not convincing to me.

Reply to point (ii): We now employ infrared mapping to trace the spatial distribution of D₂O on the AWCm surface, revealing that D₂O predominantly co-localizes with the OH AWCs and thereby providing direct evidence for OH AWCs mediated transport pathways. Therefore, we confirmed that OH AWCs mediate the transport pathways in the AWCm through MD theoretical analysis, indirect evidence from GNPs filtration experiments, and direct evidence from infrared

mapping tracing the spatial distribution of D₂O on the AWC membrane surface. Relevant descriptions have been provided in the revised manuscript.

Lines 487-493 on Page 24 in the revised manuscript.

“To more directly elucidate the role of OH AWCs in transport within the AWC membrane, Micro-FTIR mapping was employed to trace the co-localized distributions of PA, OH AWCs, and D₂O on the surface of the D₂O hydrated AWC membrane, thereby revealing that the transport pathways in the AWC membrane were mediated by the OH AWCs. In brief, Micro-FTIR was first used to characterize the functional groups on the surface of the D₂O hydrated AWC membrane, followed by mapping of the co-localized distributions of PA, OH AWCs, and D₂O based on their characteristic peaks, thereby identifying the primary regions of water molecule clustering around the OH AWCs during transport in the AWC membrane.”

Lines 252-278 on Pages 13-14 in the revised manuscript.

“MD simulations revealed that, during transport within the AWC membrane (the AWCs-PA hybrid model, OH AWCs model, and PA matrix model were shown in **Supplementary Figs. S33-S35**), water molecules were predominantly clustered in the regions of the OH AWCs (**Fig. 5A**), suggesting that OH AWCs mediate the transport pathways. Notably, the diffusion coefficient and mean squared displacement of water molecules within the OH AWCs were significantly higher than those in the PA matrix (**Fig. 5(B-C)**), suggesting that OH AWCs mediated transport was the primary contributor to the enhanced water permeance of the AWC membrane.

Gold nanoparticle (GNPs) filtration experiments further confirmed that the transport pathways in the AWC membrane were mediated by the OH AWCs. Under neutral conditions, GNPs are negatively charged and exhibit similar surface charge behavior to RO membranes (**Supplementary Fig. S36**)^{38, 39}. For GNPs deposition to occur, the drag force must overcome the electrostatic repulsion between the GNPs and the membrane surface, and therefore the resulting spatial distribution of GNPs is primarily governed by the water transport pathways³⁸. GNPs-scanning electron micrographs (GNPs-SEM) showed a uniform distribution of GNPs on the AWC-0 membrane surface (**Fig. 5D**), indicating that the PA matrix serves as the transport pathway in conventional RO membranes. However, the distribution of GNPs on the surfaces of the AWC-0.10, AWC-0.25, and AWC-0.60 membranes was more clustered compared to AWC-0 membranes (**Fig. 5(E-G)**). Furthermore, the inset SEM micrographs revealed that GNPs predominantly clustered around the OH AWCs, suggesting that OH AWCs mediate transport pathways in the AWC membrane.

Furthermore, Fourier transform infrared microspectroscopic (Micro-FTIR) mapping was used to trace the spatial distribution of D₂O on the surface of the D₂O hydrated AWC membrane, elucidating the co-localization characteristics of D₂O, PA, and the OH AWCs and probing the transport pathways in the AWC membrane. Distinct characteristic peaks of D₂O⁴⁰, PA (amide I and amide II)⁴¹, and OH AWCs (-OH)⁴² are observed on the surface of the D₂O hydrated AWC-0.25 membrane (**Fig. 5H**), based on which the co-localized mapping of PA (based on amide II

bands, **Fig. 5I**), OH AWCs (**Fig. 5J**), and D₂O (**Fig. 5K**) was established. The co-localization mapping results indicated that D₂O predominantly co-localized with the OH AWCs, more directly demonstrating that OH AWCs mediate transport pathways in the AWCm.”

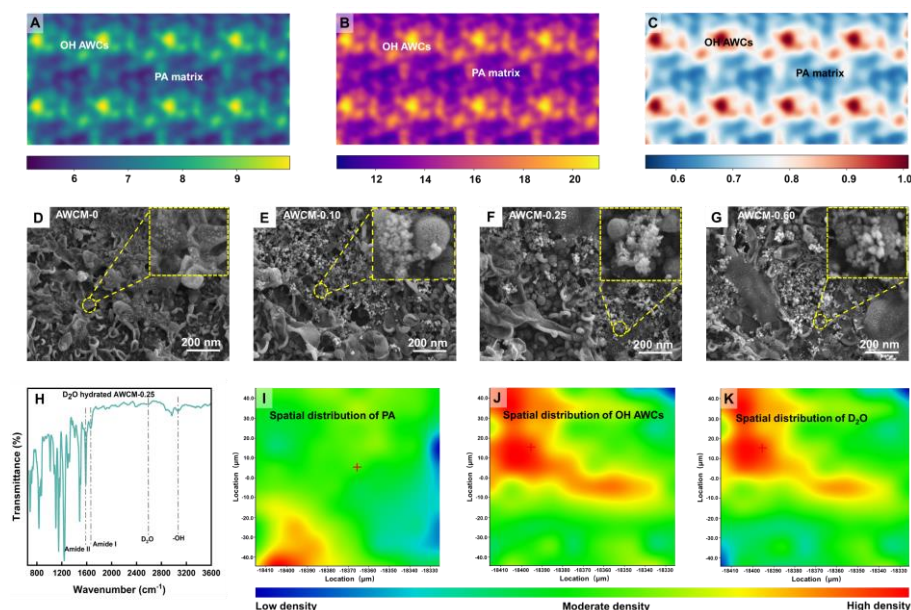


Fig. 5. The enrichment regions of water molecules on the AWCm membrane surface during transport. (A) Density distribution of water molecules in AWCm transport. (B) Diffusion coefficient of water molecules in AWCm. (C) Mean squared displacement of water molecules in AWCm. The sites of GNPs deposition on the surface of the (D) AWCm-0, (E) AWCm-0.10, (F) AWCm-0.25, and (G) AWCm-0.60 membranes (10^8 particles/mL, 25°C, 5 bar, pH=7). (H) Characteristic peaks of the D₂O hydrated AWCm-0.25 membrane surface and Fourier transform infrared microspectroscopic mapping of the co-localized distributions of (I) PA, (J) OH AWCs, and (K) D₂O.

2. Continued from the above comment, for the SiO₂@OUA channel inhibition experiments, inhibiting AWC self-assembly by co-assembling SiO₂ with OUA and then observing decreased performance (AWCM-0.25I) is interpreted as evidence that AWCs are the dominant transport pathways. However, the SiO₂@OUA aggregates introduce additional inorganic domains and also could alter the effective PA microstructure and cross-linking. These changes alone could decrease permeability and selectivity, independently of any loss of channel structures.

Our response: We agree with your incisive suggestion. We have removed the description that the experimental results of SiO₂@OUA modified RO membranes support OH AWCs mediated transport pathways. We now employ the experimental results of SiO₂@OUA modified RO membranes to support that the intact structure of OH AWCs within the PA layer is critical for maintaining the superior performance of the AWCm. Relevant descriptions have been provided in the revised manuscript.

Lines 205-236 on Pages 11-12 in the revised manuscript.

“By evaluating the separation performance of RO membranes modified after the self-assembly of nonporous SiO₂ and OUA (SiO₂@OUA, which inhibits the formation of OH AWCs), referred to as AWCM-0.25I (with “I” standing for inhibited channel formation), the role of OH AWCs was elucidated. MD simulations revealed that SiO₂ and OUA in a water/ethanol solution self-assemble into a stable aggregate via H-bonds (formed between Si-OH groups and the -OH, -C=O, and -NH groups in the OUA structure) and van der Waals interactions (arising from the alkyl chains of OUA and Si atoms) (**Fig. 4A** and **Supplementary Figs. S21-S23**), thereby inhibiting the formation of OH AWCs. Compared to AWCM-0.25, the physicochemical properties of the AWCM-0.25I membrane surface showed no significant changes, as evidenced by insignificant changes in the morphology (**Supplementary Fig. S24**), zeta potential (**Supplementary Fig. S25**), surface roughness (**Supplementary Fig. S26**) and O/N ratio (**Fig. 4B**).

The water permeance (NaCl solution) of the AWCM-0.25I decreased from 4.3 ± 0.1 to 2.8 ± 0.1 L m⁻² h⁻¹ bar⁻¹ with an increasing SiO₂ content. The salt rejection of the AWCM-0.25I decreased from $99.2 \pm 0.3\%$ to $97.9 \pm 0.4\%$ (**Fig. 4C**), and the NDMA rejection decreased from $93.9 \pm 0.1\%$ to $65.5 \pm 0.8\%$ (**Supplementary Fig. S27**). Furthermore, compared with the AWCM-0 membrane, the surface morphology, pure water permeance, and NaCl and NDMA rejection of RO membranes modified solely with SiO₂ showed no significant changes (**Supplementary Figs. S28-S31**), indicating that the intact structure of OH AWCs within the PA layer is critical for maintaining the superior separation performance of the AWCM.

Moreover, by adjusting the cross-linking degree of the PA layer (regulating PA pores), the specific role of the OH AWCs was elucidated (**Fig. 4D**). As the MPD concentration increased from 2 wt% to 6 wt%, the water permeance (NaCl solution) of the AWCM-0 was decreased by ~54.9% (**Fig. 4E**), possibly due to the formation of a higher cross-linking degree of the PA layer (reduction in the size of PA pores), as indicated by the reduced O/N ratio (**Supplementary Fig. S32**). The reduction in water permeance (NaCl solution) for AWCM-0.10, AWCM-0.25, and AWCM-0.60 was much less than that for the AWCM-0, with AWCM-0.25 showing only a ~14.8% decrease (**Fig. 4E**). Additionally, although the water permeance (NaCl solution) of AWCM-0.10, AWCM-0.25, and AWCM-0.60 decreased with increasing MPD concentration, the enhancement factor (defined as the ratio of water permeance for AWCM-0.10, AWCM-0.25, and AWCM-0.60 to that of AWCM-0 at the same MPD concentration) for water permeance increased (**Fig. 4F**). The results potentially indicated that OH AWCs mediate the transport pathways, mitigating the adverse effects of the increased cross-linking degree of the PA layer on water molecule transport.”

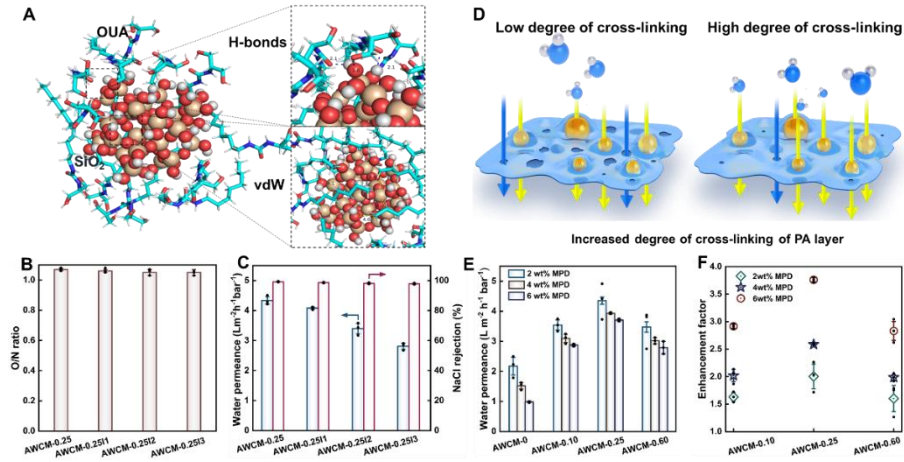


Fig. 4. Separation performance of the AWC-0.25 and AWC-0.25I (I standing for inhibited channels formation) membrane. (A) The self-assembly of nonporous SiO₂ and OUA. (B) The O/N ratio of the AWC-0.25, AWC-0.25I1, AWC-0.25I2, and AWC-0.25I3 (AWC-0.25, AWC-0.25I1, AWC-0.25I2, and AWC-0.25I3 represent the SiO₂@OUA modified RO membranes assembled at SiO₂ and OUA mass ratios of 0, 1:4, 1:2, and 1:1, respectively). (C) Water permeance (NaCl solution) and NaCl rejection of the AWC-0.25, AWC-0.25I1, AWC-0.25I2, and AWC-0.25I3 membranes [NaCl (2000 mg/L), 16 bar, 25°C]. Membrane performance under different cross-linking degree. (D) Water transport behavior in AWC with different cross-linking degree. (E) Water permeance (NaCl solution) of AWC-0, AWC-0.10, AWC-0.25, and AWC-0.60 membranes under different MPD concentrations. (F) The enhancement factor of water permeance for AWC-0.10, AWC-0.25 and AWC-0.60 compared to the AWC-0 under different MPD concentrations. Error bars in B-C and E-F represent the s.d. ($n = 3$) and data are presented as mean values $\pm$ s.d.

3. The MD simulations treat idealized OH AWCs and PA matrices separately and compare water density, diffusion coefficient, MSD, and energy barriers. However, these simulations effectively describe isolated “model” channels, not the actual composite thin-film structure formed by IP with 15–24 nm AWC particles embedded in a ~100–200 nm PA layer. There is no direct correlation that the simulated channel environment corresponds quantitatively to the real composite structure. As such, the MD results support the possibility that OH AWCs could be good transport pathways, but they do not prove that, in the real membrane, they are the dominant transport channels.

Our response: We agree with your incisive suggestion. We have now revised the description of “OH AWCs serve as primary mass transfer channels” to “OH AWCs mediated transport pathways” (i.e., good transport pathways). Our MD simulations primarily provide theoretical support for the possibility that OH AWCs mediate the transport pathways in the AWC. We now employ infrared mapping to trace the spatial distribution of D₂O on the AWC surface, revealing that D₂O predominantly co-localizes with the OH AWCs and thereby providing direct evidence for OH AWCs mediated transport pathways. Therefore, we confirmed that OH AWCs mediate the transport pathways in the AWC through MD theoretical analysis, indirect evidence from GNPs filtration experiments, and direct evidence

from infrared mapping tracing the spatial distribution of D₂O on the AWC membrane surface. Relevant descriptions have been provided in the revised manuscript.

Lines 487-493 on Page 24 in the revised manuscript.

“To more directly elucidate the role of OH AWCs in transport within the AWC membrane, Micro-FTIR mapping was employed to trace the co-localized distributions of PA, OH AWCs, and D₂O on the surface of the D₂O hydrated AWC membrane, thereby revealing that the transport pathways in the AWC membrane were mediated by the OH AWCs. In brief, Micro-FTIR was first used to characterize the functional groups on the surface of the D₂O hydrated AWC membrane, followed by mapping of the co-localized distributions of PA, OH AWCs, and D₂O based on their characteristic peaks, thereby identifying the primary regions of water molecule clustering around the OH AWCs during transport in the AWC membrane.”

Lines 252-278 on Pages 13-14 in the revised manuscript.

“MD simulations revealed that, during transport within the AWC membrane (the AWCs-PA hybrid model, OH AWCs model, and PA matrix model were shown in **Supplementary Figs. S33-S35**), water molecules were predominantly clustered in the regions of the OH AWCs (**Fig. 5A**), suggesting that OH AWCs mediate the transport pathways. Notably, the diffusion coefficient and mean squared displacement of water molecules within the OH AWCs were significantly higher than those in the PA matrix (**Fig. 5(B-C)**), suggesting that OH AWCs mediated transport was the primary contributor to the enhanced water permeance of the AWC membrane.

Gold nanoparticle (GNPs) filtration experiments further confirmed that the transport pathways in the AWC membrane were mediated by the OH AWCs. Under neutral conditions, GNPs are negatively charged and exhibit similar surface charge behavior to RO membranes (**Supplementary Fig. S36**)^{38, 39}. For GNPs deposition to occur, the drag force must overcome the electrostatic repulsion between the GNPs and the membrane surface, and therefore the resulting spatial distribution of GNPs is primarily governed by the water transport pathways³⁸. GNPs-scanning electron micrographs (GNPs-SEM) showed a uniform distribution of GNPs on the AWC-0 membrane surface (**Fig. 5D**), indicating that the PA matrix serves as the transport pathway in conventional RO membranes. However, the distribution of GNPs on the surfaces of the AWC-0.10, AWC-0.25, and AWC-0.60 membranes was more clustered compared to AWC-0 membranes (**Fig. 5(E-G)**). Furthermore, the inset SEM micrographs revealed that GNPs predominantly clustered around the OH AWCs, suggesting that OH AWCs mediate transport pathways in the AWC membrane.

Furthermore, Fourier transform infrared microspectroscopic (Micro-FTIR) mapping was used to trace the spatial distribution of D₂O on the surface of the D₂O hydrated AWC membrane, elucidating the co-localization characteristics of D₂O, PA, and the OH AWCs and probing the transport pathways in the AWC membrane. Distinct characteristic peaks of D₂O⁴⁰, PA (amide I and amide II)⁴¹, and OH AWCs (-OH)⁴² are observed on the surface of the D₂O hydrated AWC-0.25 membrane (**Fig. 5H**), based on which the co-localized mapping of PA (based on amide II

bands, **Fig. 5I**), OH AWCs (**Fig. 5J**), and D₂O (**Fig. 5K**) was established. The co-localization mapping results indicated that D₂O predominantly co-localized with the OH AWCs, more directly demonstrating that OH AWCs mediate transport pathways in the AWCm.”

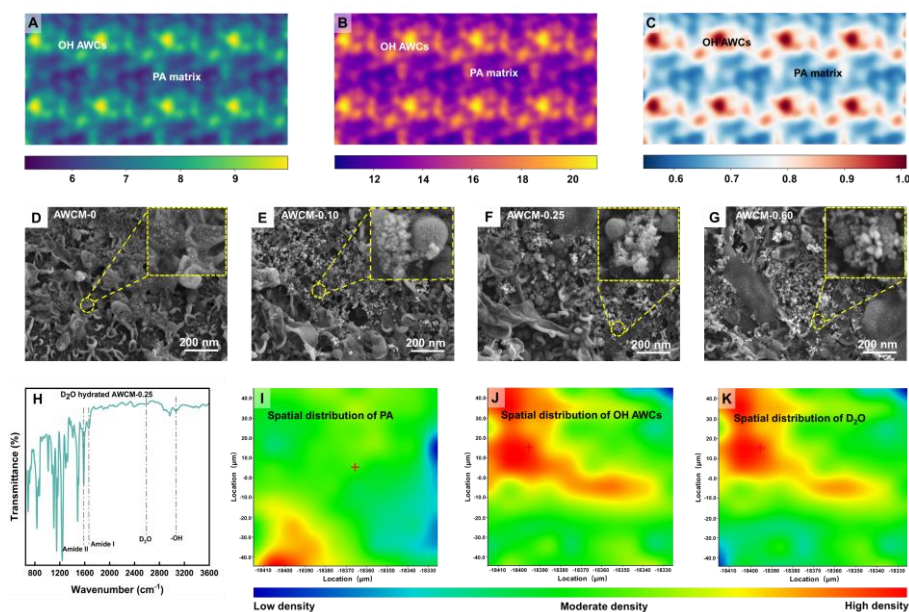


Fig. 5. The enrichment regions of water molecules on the AWCm membrane surface during transport. (A) Density distribution of water molecules in AWCm transport. (B) Diffusion coefficient of water molecules in AWCm. (C) Mean squared displacement of water molecules in AWCm. The sites of GNPs deposition on the surface of the (D) AWCm-0, (E) AWCm-0.10, (F) AWCm-0.25, and (G) AWCm-0.60 membranes (10^8 particles/mL, 25°C, 5 bar, pH=7). (H) Characteristic peaks of the D₂O hydrated AWCm-0.25 membrane surface and Fourier transform infrared microspectroscopic mapping of the co-localized distributions of (I) PA, (J) OH AWCs, and (K) D₂O.

4. Most importantly, I think that this manuscript’s novelty is limited relative to recent AWC-PA composite research. The revised introduction acknowledges that AWCs have been incorporated into PA RO membranes previously and cites several key works, as I also pointed out in the previous review round. However, the manuscript still positions itself as (1) elucidating for the first time the role of AWCs as primary mass transfer channels, and (2) the first to elucidate the mechanisms by which AWCs enhance the performance of RO membranes. In my perspective, these overstate the novelty. Several recent studies (some already cited in the rebuttal and I also commented before) have incorporated synthetic or biomimetic channels into PA membranes, discussed transport mechanisms and selectivity enhancements, and used simulations and advanced characterization to rationalize performance. In this context, the present manuscript appears as one more implementation of a similar concept (embedding a specific OH AWC into a PA layer) with performance in a comparable range, plus an extensive but still indirect mechanistic story. The incremental distinction—emphasizing NDMA and describing OH AWCs as primary mass transfer channels—does not seem sufficient to justify publication in a high-impact research journal.

Our response: Thanks for the valuable suggestion. We have removed the statements in the revised manuscript claiming (1) the first elucidation of AWCs as primary mass transfer channels and (2) the first elucidation of the mechanisms by which AWCs enhance the performance of RO membranes. To clarify the novelty of our study, we have provided comprehensive comparisons (**Table R1**, **Table R2**, and **Fig. 7**), highlighting the key differences between the representative studies cited by the reviewer and our work, as well as the distinct transport mechanism imparted by OH AWCs to RO membranes compared with the HC6 AWCs and IUP AWCs previously reported for RO membrane modification. The innovations of our study are summarized as follows:

1. Advantages of OH AWCs

At present, the incorporation of OH AWCs into RO membranes has not been reported. Moreover, compared with HC6 AWCs and IUP AWCs (containing only a small number of amino groups on the channel inner-walls), which have previously been reported for RO membrane modification, OH AWCs exhibit a higher water transport rate and abundant inner-wall -OH groups that can impart distinct transport behaviors to RO membranes. During transport through the OH AWCs, water molecules formed a greater number of H-bonds than in HC6 AWCs and IUP AWCs, while exhibiting the shortest H-bonds lifetimes and mean residence times, thereby enabling higher jumping frequencies and the lowest permeation energy barrier. For NDMA, during transport through the OH AWCs, a greater number of H-bonds were formed than in HC6 AWCs and IUP AWCs. However, the H-bonds lifetimes and mean residence times of NDMA were also higher than those in HC6 AWCs and IUP AWCs, leading to the lowest jumping frequencies and the highest permeation energy barrier in the OH AWCs. Therefore, OH AWCs can impart unexpectedly superior performance to RO membranes.

2. Performance advantages of OH AWCs modified RO membranes

Compared with RO membranes modified with HC6 AWCs and IUP AWCs reported in the literature, OH AWCs modified RO membranes not only exhibited excellent NaCl rejection, but also delivered unexpectedly ultrahigh water permeance and NDMA rejection, achieving multidimensional separation performance breakthroughs in RO membranes.

3. Mechanistic novelty

Compared with previous studies on AWCs modified RO membranes, our study confirmed that OH AWCs mediate the transport pathways through MD theoretical analysis, indirect evidence from GNPs filtration experiments, and direct evidence from Micro-FTIR mapping of the spatial distribution of D₂O on the AWCM surface, thereby providing a feasible approach for elucidating transport pathways in AWCs-based RO membranes. Furthermore, our study comprehensively elucidates that -OH induced H-bonds interactions within OH AWCs govern the transport of water molecules and NDMA in RO membranes. By comparing the H-bonds differences

associated with water and NDMA transport in OH AWCs, HC6 AWCs, and IUP AWCs, we further highlight the dominant role of H-bonds formed within AWCs in determining the performance of AWCs-based RO membranes, providing guidance for selecting suitable AWCs for the design of high-performance AWCs-based RO membranes.

Therefore, our work presents significant innovation in both RO membrane material fabrication and mechanistic elucidation. Through the incorporation of the novel channel material OH AWCs into RO membranes, substantial breakthroughs were achieved in multiple performance metrics, particularly NDMA removal and water permeance.

Table R1. Comparison between representative AWCs-based RO membrane and this work

Type	Water transport rate of AWCs	Research content	Mechanism		Study
			AWCs mediate the transport pathways	Role of channel inner-wall functional groups	
HC6 AWCs-based RO membrane	$3.8 \times 10^6 \text{ H}_2\text{O s}^{-1}$	Focus on desalination and energy consumption	/		Nat. Nanotechcnol. 16, 190, (2021)
IUP AWCs-based RO membrane	$5.79 \times 10^6 \text{ H}_2\text{O s}^{-1}$	Focus on defect-free fabrication of AWCs-based RO membranes and desalination processes	/		Nat Commun 16, 4439 (2025)
AWCM	$2.33 \times 10^8 \text{ H}_2\text{O s}^{-1}$	Focus on water permeance, desalination, and NDMA removal	Provide evidence for OH AWCs mediated transport pathways and elucidate the role of inner-wall -OH induced H-bonds in enhancing membrane performance		This work

Table R2. Performance and mechanistic comparison of RO membranes modified with HC6 AWCs, IUP AWCs, and OH-AWCs at the same concentration (0.25 wt%) in this work

Type	Pure water permeance	NaCl rejection	NDMA rejection	H-bonds interaction differences within AWCs	
				Water molecule transport	NDMA transport

HC6 AWCs modified RO	3.9 $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}\cdot\text{bar}^{-1}$	99.1%	81.2%	Fewer H-bonds and a higher water permeation energy barrier	Fewer H-bonds and a lower NDMA permeation energy barrier
IUP AWCs modified RO	4.1 $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}\cdot\text{bar}^{-1}$	99.1%	86.5%	Fewer H-bonds and a higher water permeation energy barrier	Fewer H-bonds and a lower NDMA permeation energy barrier
AWCM	4.5 $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}\cdot\text{bar}^{-1}$	99.2%	93.9%	More H-bonds and a lower water permeation energy barrier	More H-bonds and a higher NDMA permeation energy barrier

Based on the above analysis, relevant descriptions have been provided in the revised manuscript.

Lines 329-358 on Pages 17-18 in the revised manuscript.

“To further highlight the advantages of OH AWCs (containing abundant -OH groups on the inner-walls), we compared the RO membrane performance modified with OH AWCs, the I-quartet artificial water channel (HC6 AWCs)³⁰, and imidazolylethyl-ureidoethyl-phenyl AWCs (IUP AWCs)³¹(containing only a small number of amino groups on the channel inner-walls), as well as the differences in the underlying performance enhancement mechanisms. Although the NaCl rejection of RO membranes modified with OH AWCs showed no significant difference (**Supplementary Fig. S41**) compared with those modified with HC6 AWCs and IUP AWCs (the concentrations of OH AWCs, HC6 AWCs, and IUP AWCs employed for RO membrane modification were all 0.25 wt%), the OH AWCs modified RO membrane exhibited the highest pure water permeance and NDMA rejection (**Fig. 7(A-B)**). Furthermore, the transport differences of water molecules and NDMA in OH AWCs, HC6 AWCs, and IUP AWCs were further analyzed.

MD simulations (the HC6 AWCs and IUP AWCs model were shown in **Supplementary Figs. S42-S43**) revealed that, during transport through the OH AWCs, water molecules formed a greater number of H-bonds than in HC6 AWCs and IUP AWCs (**Fig. 7C**), while exhibiting the shortest H-bonds lifetimes and mean residence times of water molecules (**Fig. 7(D-E)**), thereby enabling higher jumping frequencies of water molecules and the minimal permeation energy barrier (**Fig. 7(F-G)**). For NDMA, during transport through the OH AWCs, a greater number of H-bonds were formed than in HC6 AWCs and IUP AWCs (**Fig. 7H**). However, the H-bonds lifetimes and mean residence times of NDMA were also higher than those in HC6 AWCs and IUP AWCs (**Fig. 7(I-J)**), leading to lowest jumping frequencies and the highest permeation energy barrier in the OH AWCs (**Fig. 7(K-L)**). The results indicated that the transport performance of both water molecules and NDMA in the OH AWCs surpassed that in HC6 AWCs and IUP AWCs, primarily attributed to H-bonds interactions induced by the

abundant polar -OH groups on the inner-walls of the OH AWCs. Therefore, incorporation of OH AWCs into RO membranes can confer unexpectedly superior performance, enabling multidimensional breakthroughs in membrane performance. Furthermore, the results suggested that differences in the functional group characteristics of the inner-walls of AWCs conferred distinct transport behaviors to water molecules and solutes, resulting in different transport properties of RO membranes. This study offers valuable design principles for selecting AWCs with strongly polar functional groups on the channel walls for RO membrane modification to enhance H-bonds interactions during transport in high-performance AWCs-based RO membranes.”

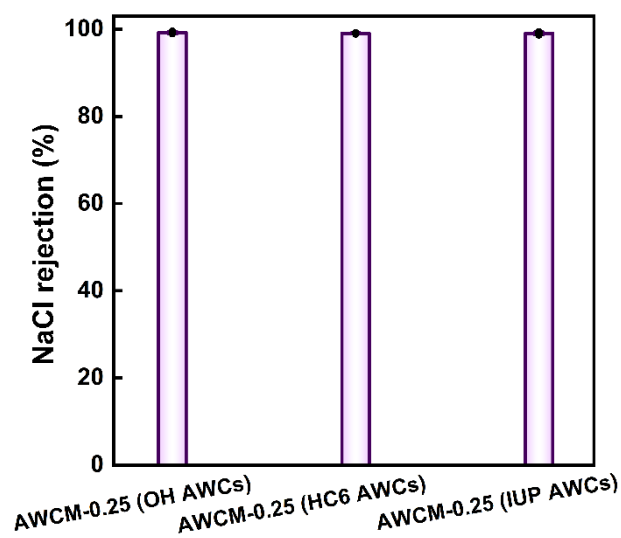


Fig. S41. NaCl rejection of RO membranes modified with OH AWCs, HC6 AWCs, and IUP AWCs. Error bars represent the s.d. ($n = 3$) and data are presented as mean values $\pm$ s.d.

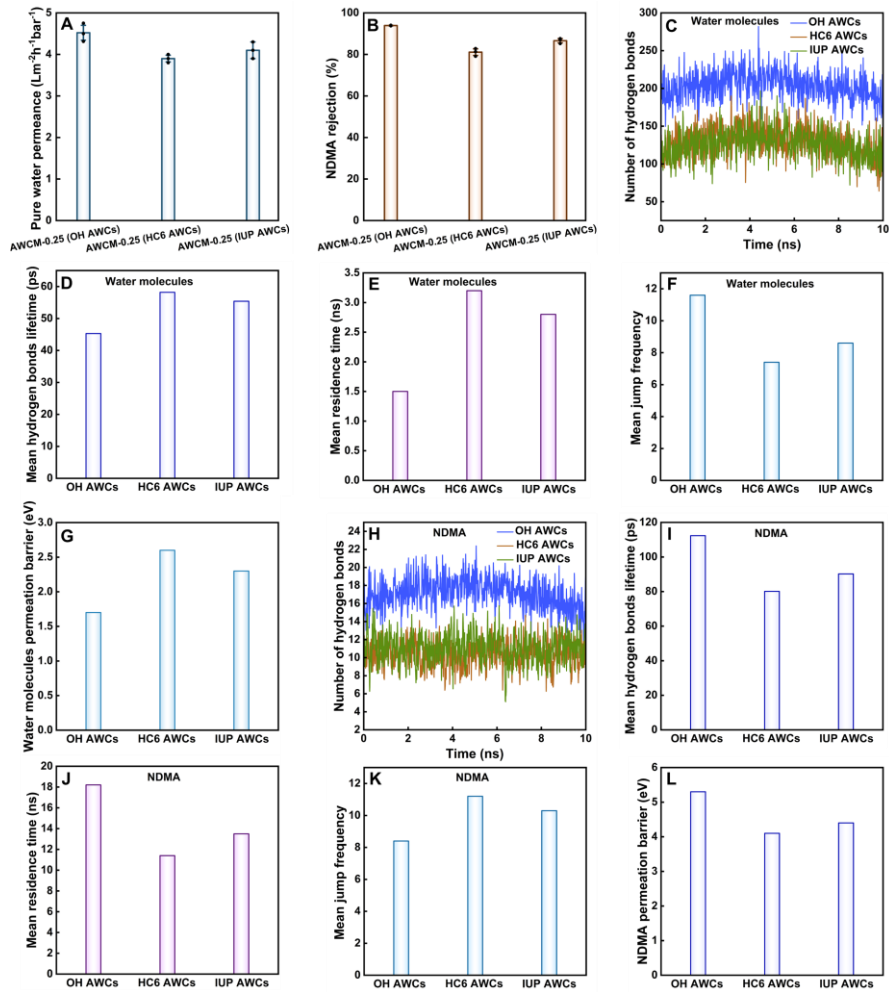


Fig. 7. Comparison of RO membrane performance modified with different AWCs and the transport characteristics of water molecules and NDMA within these AWCs. (A) Pure water permeance and (B) NDMA rejection of RO membranes modified with OH AWCs, HC6 AWCs, and IUP AWCs. Comparison of (C) the number of H-bonds formed during water transport in OH AWCs, HC6 AWCs, and IUP AWCs, as well as (D) H-bonds lifetimes, (E) mean residence times of water molecules, and (F) mean jumping frequencies. (G) Permeation energy barriers of water molecules in different AWCs. Comparison of (H) the number of H-bonds formed during NDMA transport in OH AWCs, HC6 AWCs, and IUP AWCs, as well as (I) H-bonds lifetimes, (J) mean residence times of NDMA, and (K) mean jumping frequencies. (L) Permeation energy barriers of NDMA in different AWCs. Error bars in A and B represent the s.d. ($n = 3$) and data are presented as mean values $\pm$ s.d.

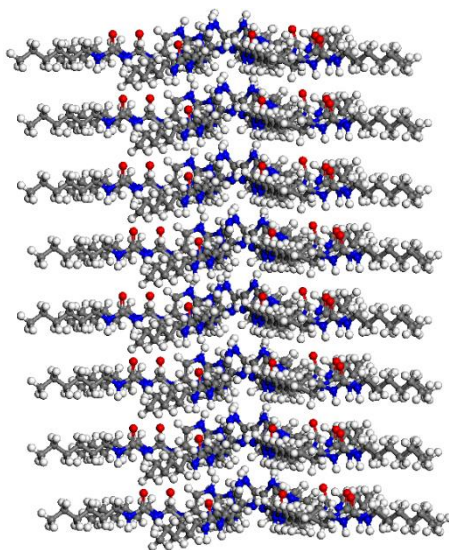


Fig. S42. Structure of HC6 AWCs.

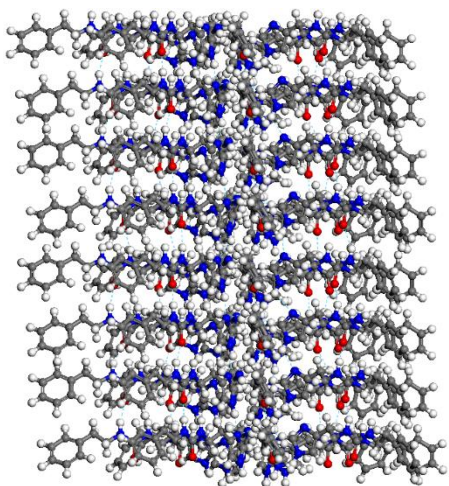


Fig. S43. Structure of IUP AWCs.

Reviewer #3:

The authors have made commendable efforts in addressing the major concerns raised in the first round, and the revised manuscript reflects significant improvement. However, I would like to raise a point regarding the terminology used in the XRD analysis. The authors state in line 160: "Compared with the pristine RO membrane, the AWCM exhibited a red shift in the diffraction peak (Supplementary Fig. S20), which was attributed to the incorporation of OH AWCs into the PA layer." The use of the term "red shift" in the context is non-standard and potentially confusing, as it is typically reserved for optical spectroscopy. I suggest the authors revise this sentence to reflect standard terminology.

Our response: Thanks for the incisive suggestion. We have revised the description from "Compared with the pristine RO membrane, the AWCM exhibited a red shift in the diffraction peak, which was attributed to the incorporation of OH AWCs into the PA layer" to "Compared with the pristine RO membrane, the AWCM exhibited a shift in the diffraction peak, which was attributed to the incorporation of OH AWCs into the PA layer.". Relevant descriptions have been provided in the revised manuscript.

Lines 152-154 on Page 8 in the revised manuscript.

"Compared with the pristine RO membrane, the AWCM exhibited a shift in the diffraction peak (**Supplementary Fig. S16**), which was attributed to the incorporation of OH AWCs into the PA layer."

Reviewer #4:

This work demonstrates a polyamide membrane incorporated with hydroxyl artificial water channels (OH-AWCs) for the effective removal of N-nitrosodimethylamine (NDMA). While the separation performance is impressive, the mechanistic evidence supporting water transport through the OH-AWCs remains insufficient. The primary concerns, also noted by other reviewers, center on the validation of the proposed water transport pathway and the demonstration of novelty relative to other AWCs. To address these issues and meet the high standard of Nature Communications, I recommend the authors provide additional experimental and theoretical evidence.

Our response: Thanks for dedicating your time and providing a thoughtful review. In response to your incisive suggestions, we have carefully incorporated them into the revised manuscript. Detailed point-by-point responses are provided below.

1. Elucidating the water transport pathway through OH-AWCs: The current Raman mapping data lack the spatial resolution to conclusively demonstrate water movement through the channels. Although control experiments offer indirect support, direct evidence is needed. A possible approach would be to employ isotopic labeling. By permeating deuterated water (D_2O) through the membrane and tracking its distribution, the primary transport pathway could be visualized. For instance, D_2O exhibits a characteristic IR peak near 2540 cm^{-1} (Chem. Commun., 2015, 51, 3251-3254), which could be mapped using IR spectroscopic microscopy or related techniques. If D_2O is found to co-localize with the OH-AWCs rather than the bulk polyamide matrix, this would provide strong direct evidence for channel-mediated transport.

Our response: Based on your incisive suggestion, we have now employed infrared mapping to trace the spatial distribution of D_2O on the AWCM surface, revealing that D_2O predominantly co-localizes with the OH AWCs and thereby providing direct evidence that the transport pathways are mediated by the OH AWCs. Relevant descriptions have been provided in the revised manuscript.

Lines 487-493 on Page 24 in the revised manuscript.

“To more directly elucidate the role of OH AWCs in transport within the AWCM membrane, Micro-FTIR mapping was employed to trace the co-localized distributions of PA, OH AWCs, and D_2O on the surface of the D_2O hydrated AWCM, thereby revealing that the transport pathways in the AWCM were mediated by the OH AWCs. In brief, Micro-FTIR was first used to characterize the functional groups on the surface of the D_2O hydrated AWCM, followed by mapping of the co-localized distributions of PA, OH AWCs, and D_2O based on their characteristic peaks, thereby identifying the primary regions of water molecule clustering around the OH AWCs during transport in the AWCM.”

Lines 252-278 on Pages 13-14 in the revised manuscript.

“MD simulations revealed that, during transport within the AWCM (the AWCs-PA hybrid model, OH AWCs model, and PA matrix model were shown in **Supplementary Figs. S33-S35**), water molecules were predominantly clustered in the regions of the OH AWCs (**Fig. 5A**), suggesting that OH AWCs mediate the transport pathways. Notably, the diffusion coefficient and mean squared displacement of water molecules within the OH AWCs were significantly higher than those in the PA matrix (**Fig. 5(B-C)**), suggesting that OH AWCs mediated transport was the primary contributor to the enhanced water permeance of the AWCM.

Gold nanoparticle (GNPs) filtration experiments further confirmed that the transport pathways in the AWCM were mediated by the OH AWCs. Under neutral conditions, GNPs are negatively charged and exhibit similar surface charge behavior to RO membranes (**Supplementary Fig. S36**)^{38, 39}. For GNPs deposition to occur, the drag force must overcome the electrostatic repulsion between the GNPs and the membrane surface, and therefore the resulting spatial distribution of GNPs is primarily governed by the water transport pathways³⁸. GNPs-scanning electron micrographs (GNPs-SEM) showed a uniform distribution of GNPs on the AWCM-0 membrane surface (**Fig. 5D**), indicating that the PA matrix serves as the transport pathway in conventional RO membranes. However, the distribution of GNPs on the surfaces of the AWCM-0.10, AWCM-0.25, and AWCM-0.60 membranes was more clustered compared to AWCM-0 membranes (**Fig. 5(E-G)**). Furthermore, the inset SEM micrographs revealed that GNPs predominantly clustered around the OH AWCs, suggesting that OH AWCs mediate transport pathways in the AWCM.

Furthermore, Fourier transform infrared microspectroscopic (Micro-FTIR) mapping was used to trace the spatial distribution of D₂O on the surface of the D₂O hydrated AWCM, elucidating the co-localization characteristics of D₂O, PA, and the OH AWCs and probing the transport pathways in the AWCM. Distinct characteristic peaks of D₂O⁴⁰, PA (amide I and amide II)⁴¹, and OH AWCs (-OH)⁴² are observed on the surface of the D₂O hydrated AWCM-0.25 membrane (**Fig. 5H**), based on which the co-localized mapping of PA (based on amide II bands, **Fig. 5I**), OH AWCs (**Fig. 5J**), and D₂O (**Fig. 5K**) was established. The co-localization mapping results indicated that D₂O predominantly co-localized with the OH AWCs, more directly demonstrating that OH AWCs mediate transport pathways in the AWCM.”

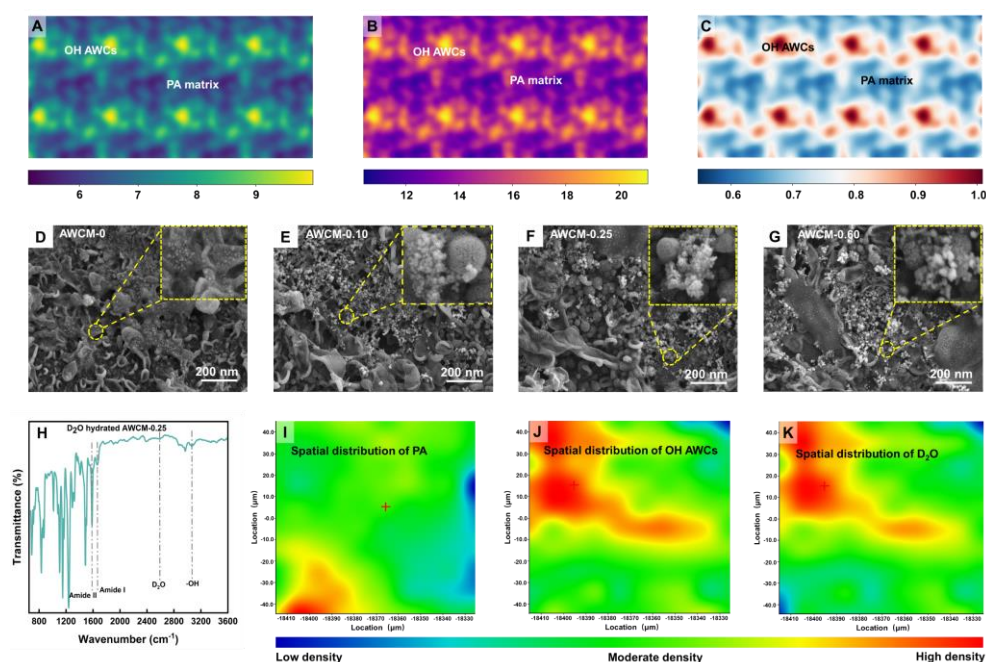


Fig. 5. The enrichment regions of water molecules on the AWC membrane surface during transport. (A) Density distribution of water molecules in AWC transport. (B) Diffusion coefficient of water molecules in AWC. (C) Mean squared displacement of water molecules in AWC. The sites of GNPs deposition on the surface of the (D) AWC-0, (E) AWC-0.10, (F) AWC-0.25, and (G) AWC-0.60 membranes (10^8 particles/mL, 25°C , 5 bar, $\text{pH}=7$). (H) Characteristic peaks of the D_2O hydrated AWC-0.25 membrane surface and Fourier transform infrared microspectroscopic mapping of the co-localized distributions of (I) PA, (J) OH AWCs, and (K) D_2O .

2. *Substantiating the novelty of OH-AWCs: The manuscript's central claim hinges on the unique efficacy of the hydroxyl-aligned channels. To better underscore this novelty, I recommend incorporating a comparative study. The authors could fabricate PA membranes with other, well-characterized AWCs and evaluate their performance alongside the OH-AWC variant. Additionally, molecular dynamics (MD) simulations comparing water transport, hydrogen-bonding dynamics, and rejection across different AWC types would greatly strengthen the argument. Such a combined experimental and theoretical comparison would not only clarify the advantage of OH-AWCs but also offer valuable design principles for future channel-based membranes.*

Our response: Thanks for the incisive suggestion. We compared the performance of RO membranes modified with OH AWCs, HC6 AWCs, and IUP AWCs, and further analyzed the H-bonds induced transport differences of water molecules and NDMA in different AWCs through MD simulations. This result highlights the unique advantages of OH AWCs in mass transfer and the novelty of our work.. Relevant descriptions have been provided in the revised manuscript.

Lines 312-316 on Page 16 in the revised manuscript.

“For NDMA, during transport through the OH AWCs, a greater number of H-bonds were formed than in the PA matrix (**Supplementary Fig. S37**), and these H-bonds exhibited longer lifetimes (**Supplementary Fig. S38**), leading to prolonged residence times and lower jumping frequencies (**Supplementary Figs. S39-S40**), consequently OH AWCs mediated transport pathways conferred a higher permeation energy barrier for NDMA (**Fig. 6I**) and superior rejection in the AWCs.”

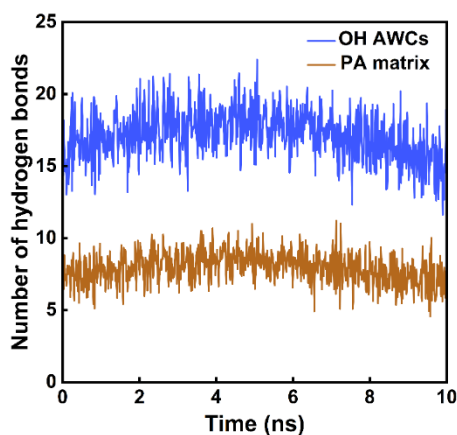


Fig. S37. Number of hydrogen bonds formed during NDMA transport in OH AWCs and the PA matrix.

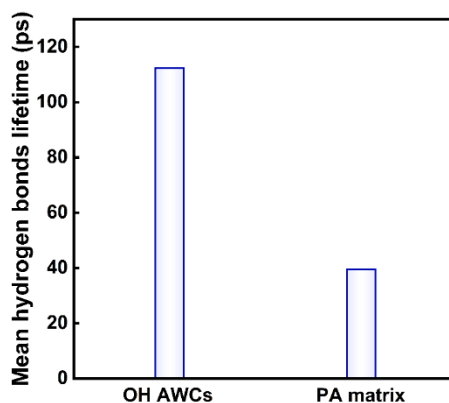


Fig. S38. Mean hydrogen bonds lifetime during NDMA transport in OH AWCs and the PA matrix.

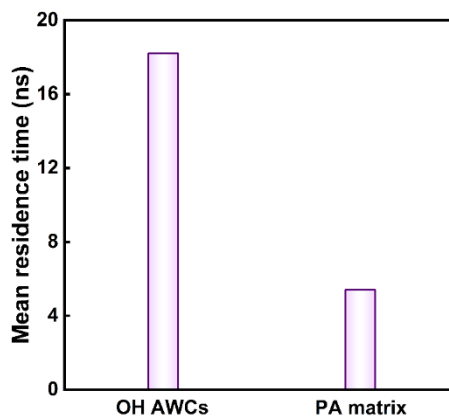


Fig. S39. Mean residence time of NDMA during transport in OH AWCs and the PA matrix.

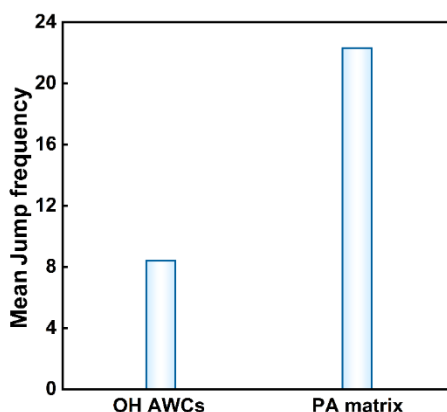


Fig. S40. Mean jumping frequency of NDMA during transport in OH AWCs and the PA matrix.

Lines 329-358 on Pages 17-18 in the revised manuscript.

“To further highlight the advantages of OH AWCs (containing abundant -OH groups on the inner-walls), we compared the RO membrane performance modified with OH AWCs, the I-quartet artificial water channel (HC6 AWCs)³⁰, and imidazolylethyl-ureidoethyl-phenyl AWCs (IUP AWCs)³¹(containing only a small number of amino groups on the channel inner-walls), as well as the differences in the underlying performance enhancement mechanisms. Although the NaCl rejection of RO membranes modified with OH AWCs showed no significant difference (**Supplementary Fig. S41**) compared with those modified with HC6 AWCs and IUP AWCs (the concentrations of OH AWCs, HC6 AWCs, and IUP AWCs employed for RO membrane modification were all 0.25 wt%), the OH AWCs modified RO membrane exhibited the highest pure water permeance and NDMA rejection (**Fig. 7(A-B)**). Furthermore, the transport differences of water molecules and NDMA in OH AWCs, HC6 AWCs, and IUP AWCs were further analyzed.

MD simulations (the HC6 AWCs and IUP AWCs model were shown in **Supplementary Figs. S42-S43**) revealed that, during transport through the OH AWCs, water molecules formed a greater number of H-bonds than in HC6 AWCs and IUP AWCs (**Fig. 7C**), while exhibiting the shortest H-bonds lifetimes and mean residence times of water molecules (**Fig. 7(D-E)**), thereby enabling higher jumping frequencies of water molecules and the minimal permeation energy barrier (**Fig. 7(F-G)**). For NDMA, during transport through the OH AWCs, a greater number of H-bonds were formed than in HC6 AWCs and IUP AWCs (**Fig. 7H**). However, the H-bonds lifetimes and mean residence times of NDMA were also higher than those in HC6 AWCs and IUP AWCs (**Fig. 7(I-J)**), leading to lowest jumping frequencies and the highest permeation energy barrier in the OH AWCs (**Fig. 7(K-L)**). The results indicated that the transport performance of both water molecules and NDMA in the OH AWCs surpassed that in HC6 AWCs and IUP AWCs, primarily attributed to H-bonds interactions induced by the abundant polar -OH groups on the inner-walls of the OH AWCs. Therefore, incorporation of OH AWCs into RO membranes can confer unexpectedly superior performance, enabling

multidimensional breakthroughs in membrane performance. Furthermore, the results suggested that differences in the functional group characteristics of the inner-walls of AWCs conferred distinct transport behaviors to water molecules and solutes, resulting in different transport properties of RO membranes. This study offers valuable design principles for selecting AWCs with strongly polar functional groups on the channel walls for RO membrane modification to enhance H-bonds interactions during transport in high-performance AWCs-based RO membranes.”

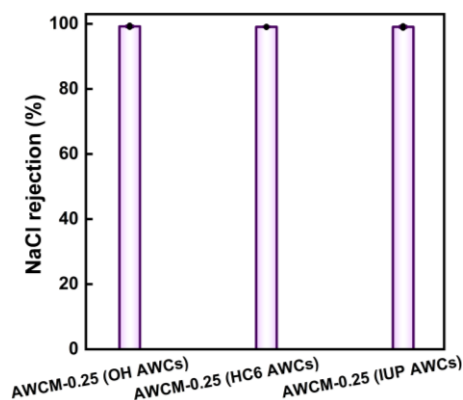


Fig. S41. NaCl rejection of RO membranes modified with OH AWCs, HC6 AWCs, and IUP AWCs. Error bars represent the s.d. ($n = 3$) and data are presented as mean values $\pm$ s.d.

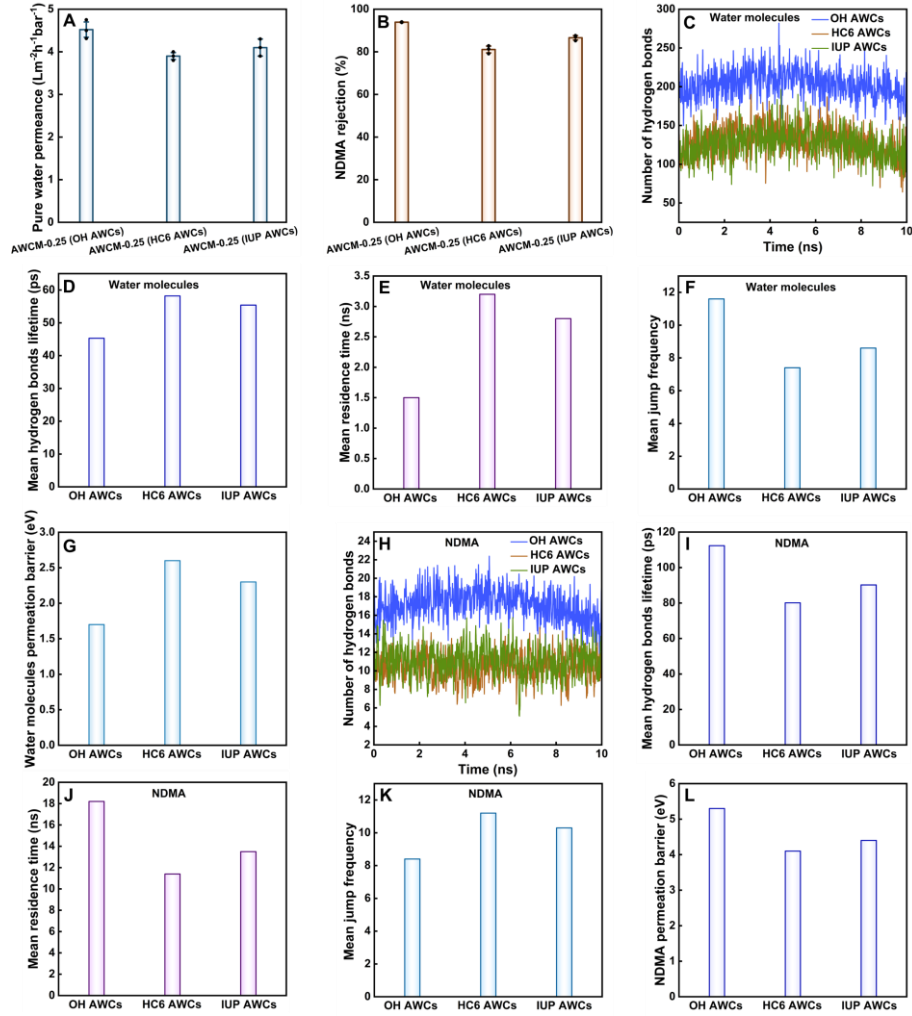


Fig. 7. Comparison of RO membrane performance modified with different AWCs and the transport characteristics of water molecules and NDMA within these AWCs. (A) Pure water permeance and (B) NDMA rejection of RO membranes modified with OH AWCs, HC6 AWCs, and IUP AWCs. Comparison of (C) the number of H-bonds formed during water transport in OH AWCs, HC6 AWCs, and IUP AWCs, as well as (D) H-bonds lifetimes, (E) mean residence times of water molecules, and (F) mean jumping frequencies. (G) Permeation energy barriers of water molecules in different AWCs. Comparison of (H) the number of H-bonds formed during NDMA transport in OH AWCs, HC6 AWCs, and IUP AWCs, as well as (I) H-bonds lifetimes, (J) mean residence times of NDMA, and (K) mean jumping frequencies. (L) Permeation energy barriers of NDMA in different AWCs. Error bars in A and B represent the s.d. ($n = 3$) and data are presented as mean values $\pm$ s.d.

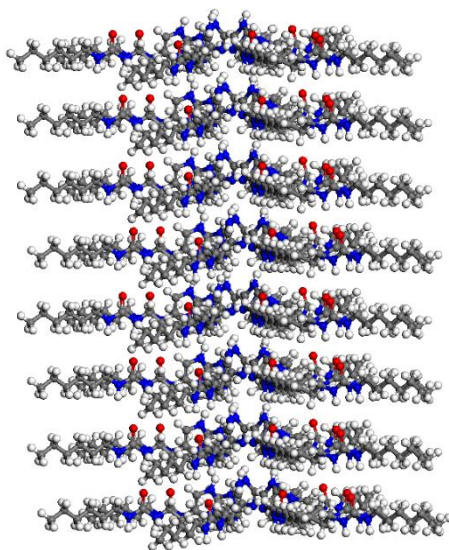


Fig. S42. Structure of HC6 AWCs.

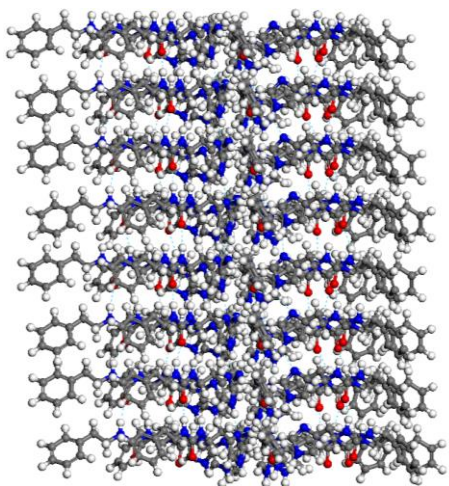


Fig. S43. Structure of IUP AWCs.

Response to Reviewer Comments and List of Changes Made in the Manuscript

Reviewer #4:

I appreciate the authors' efforts to accept my suggestions on the IR mapping to track the water pathway. Now, the mapping in Fig. 5 J-K have shown the spatial correlation between the artificial channel and water pathway. I believe it can solve the related concerns raised by the other reviewers. My only suggestion is to improve the Fig. 5H, in particular at D₂O region on the following points:

Our response: Thanks for dedicating your time and providing a thoughtful review. We have carefully revised our manuscript based on the suggestions. Point-by-point responses are provided below.

1. Signal-to-noise ratio (SNR): They may extend the accumulation times for spectra collection, or reduce the thickness of membrane to suppress the signal from PA matrix. They may also convert the transmittance to absorbance unite if the SNR appears better.

Our response: Based on your incisive suggestion, to reduce interference from the PA matrix and improve the signal-to-noise ratio, we extended the accumulation time to 128 scans for spectral collection and converted the transmittance spectra into absorbance spectra. The results showed that the pure PA matrix exhibited no peak at the characteristic wavenumber of D₂O, confirming no spectral interference from the PA matrix with the D₂O signal (**Fig. 5H**). Relevant description has been provided in the revised manuscript.

Lines 499-500 on Page 25 in the revised manuscript.

“To improve the signal-to-noise ratio, the spectral data were collected by accumulating 128 scans.”

Lines 277-278 on Page 14 in the revised manuscript.

“Notably, the pure PA matrix exhibited no peak at the characteristic wavenumber of D₂O, confirming no spectral interference from the PA matrix with the D₂O signal (**Fig. 5H**).”

Lines 286-289 on Page 15 in the revised manuscript.

“(H) Characteristic peaks of the pure PA matrix and the D₂O hydrated AWCM-0.25 membrane, and Fourier-transform infrared microspectroscopic mapping of the co-localized distributions of (I) PA, (J) OH AWCs, and (K) D₂O on the D₂O hydrated AWCM-0.25 membrane surface.”

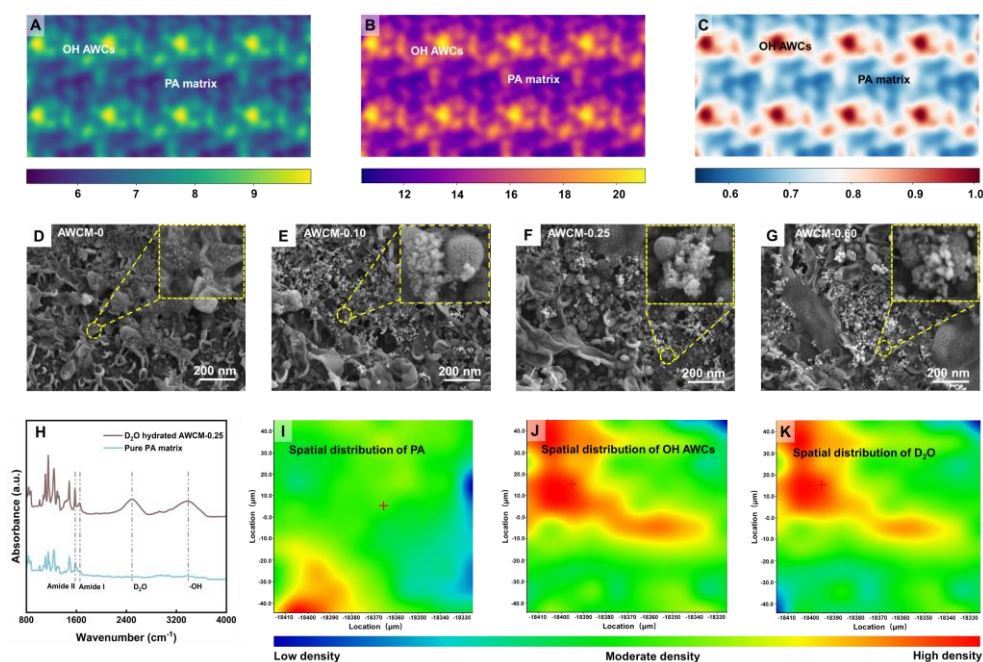


Fig. 5. The enrichment regions of water molecules on the AWC membrane surface during transport. (A) Density distribution of water molecules in AWC transport. (B) Diffusion coefficient of water molecules in AWC. (C) Mean squared displacement of water molecules in AWC. The sites of GNPs deposition on the surface of the (D) AWC-0, (E) AWC-0.10, (F) AWC-0.25, and (G) AWC-0.60 membranes (10^8 particles/mL, 25°C , 5 bar, $\text{pH}=7$). (H) Characteristic peaks of the pure PA matrix and the D_2O hydrated AWC-0.25 membrane, and Fourier-transform infrared microspectroscopic mapping of the co-localized distributions of (I) PA, (J) OH AWCs, and (K) D_2O on the D_2O hydrated AWC-0.25 membrane surface.

2. Controlling baseline: The author can also add the IR spectra of pure PA matrix to check if there is an interference from PA matrix.

Our response: We agree with your comment. We have now added the FTIR spectra of the pure PA matrix. The results showed that the pure PA matrix exhibited no peak at the characteristic wavenumber of D_2O , confirming no spectral interference from the PA matrix with the D_2O signal (**Fig. 5H**). Relevant description has been provided in the revised manuscript.

Lines 277-278 on Page 14 in the revised manuscript.

“Notably, the pure PA matrix exhibited no peak at the characteristic wavenumber of D_2O , confirming no spectral interference from the PA matrix with the D_2O signal (**Fig. 5H**).”

Lines 286-289 on Page 15 in the revised manuscript.

“(H) Characteristic peaks of the pure PA matrix and the D_2O hydrated AWC-0.25 membrane, and Fourier-transform infrared microspectroscopic mapping of the co-localized distributions of (I) PA, (J) OH AWCs, and (K) D_2O on the D_2O hydrated AWC-0.25 membrane surface.”

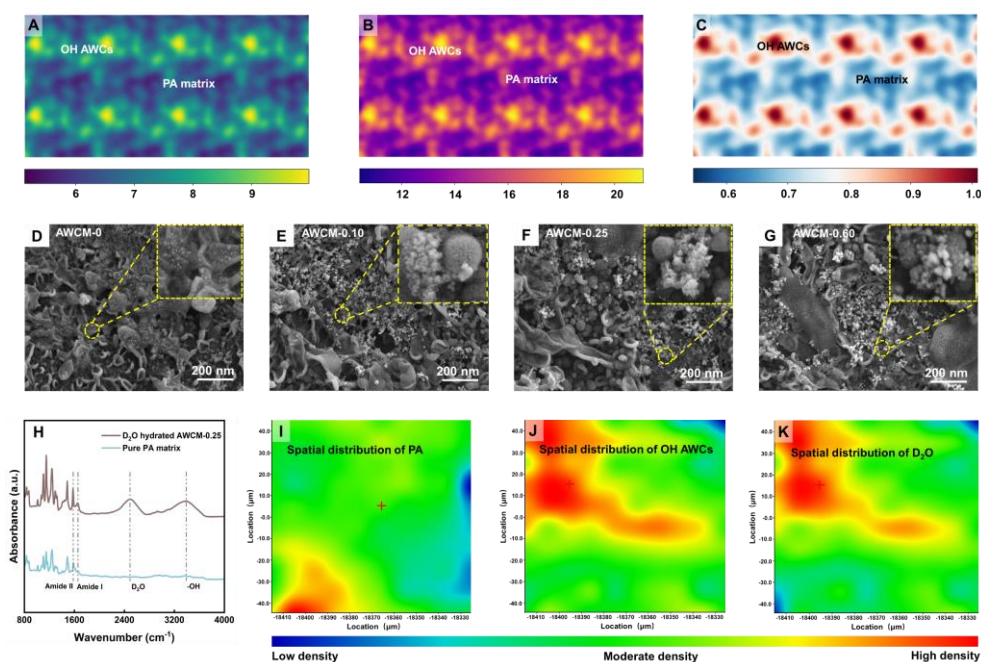


Fig. 5. The enrichment regions of water molecules on the AWC membrane surface during transport. (A) Density distribution of water molecules in AWC transport. (B) Diffusion coefficient of water molecules in AWC. (C) Mean squared displacement of water molecules in AWC. The sites of GNPs deposition on the surface of the (D) AWC-0, (E) AWC-0.10, (F) AWC-0.25, and (G) AWC-0.60 membranes (10^8 particles/mL, 25°C, 5 bar, pH=7). (H) Characteristic peaks of the pure PA matrix and the D₂O hydrated AWC-0.25 membrane, and Fourier-transform infrared microspectroscopic mapping of the co-localized distributions of (I) PA, (J) OH AWCs, and (K) D₂O on the D₂O hydrated AWC-0.25 membrane surface.

3. *Experimental parameters: More details on the IR mapping can be added in the method section to establish an example for the other investigators on artificial water channel. For instance, the intruement brand, detector, exposure/accumulation time, object etc.*

Our response: We agree with your comment. We have now added more details on the testing procedure for Micro-FTIR mapping to the methods section. Specifically, Micro-FTIR mapping was performed using a Thermo Scientific Nicolet iN10 infrared microscope equipped with a DLaTGS detector, a 15× objective, and a 15× condenser. The spectra were collected over the wavenumber range of 4000-675 cm^{-1} at a spectral resolution of 8 cm^{-1} . To improve the signal-to-noise ratio, the spectral data were collected by accumulating 128 scans. Relevant description has been provided in the revised manuscript.

Lines 496-500 on Pages 24-25 in the revised manuscript.

“Specifically, Micro-FTIR mapping was performed using a Thermo Scientific Nicolet iN10 infrared microscope equipped with a DLaTGS detector, a 15× objective, and a 15× condenser. The spectra were collected over the wavenumber range of 4000-675 cm^{-1} at a spectral resolution of 8 cm^{-1} . To improve the signal-to-noise ratio, the spectral data were collected by accumulating 128 scans.”

The authors polyamide RO membranes embedded with a previously reported artificial water channels with aligned hydroxyl groups to address a critical limitation in RO membranes: the poor rejection of small, uncharged, and carcinogenic molecules such as NDMA. Incorporating OH AWCs into the PA layer leads to over 30% improvement in NDMA rejection compared to the unmodified PA membrane, while maintaining high water permeance. The resulting membrane surpasses the conventional selectivity–permeability trade-off observed in RO membranes, potentially reducing the need for downstream treatment processes to remove NDMA. The role of AWCs is supported by a combination of experimental characterization and molecular dynamics (MD) simulations. This study addresses an important challenge in water reuse and proposes a promising design strategy. However, several critical concerns remain before I can recommend for publication. These issues should be addressed to strengthen the validity of the core claims.

1. The central claim is that OH AWCs are responsible for the enhanced NDMA rejection. While the authors present control membranes with SiO₂ to inhibit AWC activity and observe reductions in water permeance and NaCl rejection, they do not report NDMA rejection for these inhibited membranes. Since NDMA rejection is the focal point of the study, it is essential to demonstrate that removing AWC functionality also compromises NDMA exclusion.
2. The inhibitory control membranes contain both SiO₂ and OUA. However, it is unclear whether SiO₂ alone could impact PA morphology or transport properties. The absence of a control membrane prepared with SiO₂ alone makes it difficult to isolate the specific role of SiO₂ as an inhibitor. Including this control would clarify whether the observed changes in transport are due to inhibited AWC formation or other effects from the SiO₂ additive.
3. The WAXS data demonstrating subnanometer pore formation were apparently collected from self-assembled OH AWC aggregates in isolation, not from the final membranes. Given that the AWCs are integrated during interfacial polymerization—a process involving organic solvents and chemical crosslinking—the assumption that OH AWCs retain their channel structure within the PA matrix requires experimental validation. The authors should discuss whether such structural integrity can realistically be retained and provide supporting evidence.
4. The authors interpret the O/N ratio from XPS as evidence of successful AWC incorporation. However, if this ratio is taken as quantitative, one would expect a monotonic increase in O/N ratio with higher AWC loading. The authors should clarify.