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Highlights from the Faraday Discussion on Artificial Water Channels, Glasgow, UK.

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Faraday Discussions have a 100 year old format: each speaker has to submit a full paper in advance and then presents a 5-minute summary of the work at the conference. Each session of lectures is followed by questions and intense discussions from the delegates, who have had the opportunity to read the papers in advance and prepare their questions.



Figure 1. The Technology & Innovation Centre, University of Strathclyde, Glasgow, UK.

This gave the participants the rare opportunity of meeting over the boundaries of the disciplines, and the possibility of finding a common language to discuss scientific aspects from various perspectives, which lies somewhere between traditional conference questions and live, non-anonymous, peer-review. For the vast majority of delegates, this was their first time attending a Faraday Discussion. It was a unique opportunity to discuss high level projects and results with the very people that are developing them.



Figure 2. Participants to the Faraday Discussion on Artificial Water Channels

The Faraday Discussion on Artificial Water Channels was the first of its kind organized on a recently conceived field¹⁻⁴ and was held from 25-27 June 2018 at the Technology & Innovation Centre at the University of Strathclyde. (Fig. 1). It was a nice opportunity to meet promising younger researchers and the world's leading scientists from 21 countries from all corners of the world (Fig. 2) and to have fruitful discussions on the growing importance of Artificial Water Channels. The aim of the discussion was to create conditions for exchange and interaction between the different fields relating to water transport: Biological porins, Artificial water channels, carbon nanotubes or graphene-based materials, and membranes for desalination and water treatment. Despite the imaginative work by chemists to produce sophisticated synthetic architectures confining complex water clusters, most water-channel based work in the past was conducted with natural proteins, used as the selectivity components, embedded in the diverse arrays. A recent step was to construct simpler compounds that maintain high-water transport activity and lead to fully synthetic artificial water channels. Moving to simple water-channel systems offers a chance to better understand mechanistic and structural behaviors that might parallel those in biomolecular systems, and toward the development of desalination membranes embedding artificial channels for highly selective water transport.



Figure 3. Professor Peter Pohl from Johannes Kepler University Linz presenting his Introductory Lecture on Single-file transport of water through membrane channels.

Following a welcome and introduction to the format by Dr. Mihail Barboiu FRSC (CNRS Montpellier, France), the Chair of the Scientific Committee, and the RSC's Publishing Editors for the event, Suzanne Howson and Sarah Whitbread, the program began with a talk by Professor Peter Pohl from the Johannes Kepler University (JKU), Linz on "Single-file transport of water through membrane channels" (Fig. 3).⁵ This talk discussed the evolution of thinking on this topic starting with a discussion of the simple theory of water diffusion in pores proposed by Alan Finkelstein that proposed that osmotic single channel water permeability could be expected to be inversely proportional to the length of the channel.⁶ The talk then showed, by citing data from extensive experiments conducted in the Pohl group and other labs, that this was not true and that a more direct correlation can be made between the number of hydrogen bonding sites within water channels and single channel permeability and further to the relationship between single channel permeability and Gibbs activation energy barrier for each channel. Another highlight of the talk was the careful experimental procedure that Pohl and co-workers have developed to obtain both single channel water permeability and ion permeability for water and ion channels. While this has not been adopted widely in the water channel field as of yet, the general consensus from the discussions that followed was that such a procedure was necessary for the development of the artificial and biological water channels field. This lecture then allowed the leading competences from different scientific areas to discuss Artificial Water Channels during four mixed sessions.

Session 1 "Structure and function of natural proteins for water transport".

The talks in this session dealt with water transport in biological transport proteins and its measurement (Fig. 4).



Figure 4. From the left to the right, the Introductory Lecturer, Professor Peter Pohl together with the speakers of the session 1: Dr. Andreas Horner, Dr. Susanna Törnroth-Horsefield and Dr. Aude Sadet together with the chair of the Session 1 Prof. Manish Kumar and the Chair of the Faraday Discussion Dr. Mihail Barboiu.

Dr. Susana Törnroth-Horsefield from Lund University discussed her collaborative work conducted with Stefan Kreida, Jennifer Virginia Roche, Caroline Olsson, and Sara Linse, on protein-protein interactions regulating aquaporin function.⁷ She presented on the Aquaporin-0 (AQP0) calmodulin system that modulates permeability by protein-protein interactions as well as the AQP2-LIP5 system that modulates membrane permeability by affecting trafficking of AQP-rich vesicles to and from the membrane. Dr. Andreas Horner from JKU, presenting on collaborative work with Professor Peter Pohl, followed up on single file transport in biological channels and reported on recent experiments on determining the effect of charge of residues at the entrance of water channels on single channel permeability. He concluded that positive charges at the entrance of channels boost water transport while negative charges do not.⁸ Dr. Aude Sadet from the University of Bucharest, then presented collaborative work with Viorel Nastasa, Cristina Stavarache, Anamaria Hanganu, Dr. Andina Coroaba, Dr. Alina Nicolescu, Dr. Calin Deleanu, and Prof. Paul Vasos on an intriguing hyperpolarized NMR based proposal to evaluate water transport across vesicular membranes mediated by channels.⁹ This technique holds promise in the future but is somewhat limited at present due to the sampling interval minimum of 100 ms to 1 s. After the presentations there was a lively discussion, the main upshot of which was a consensus on the need to develop standard ways of measuring, reporting, and comparing single channel water and ion permeabilities. Further, there exists a need to develop orthogonal techniques to measure water and ion permeabilities that go beyond the current stopped flow and electrophysiological techniques. Finally, thinking about trafficking and dynamic membrane integration as an additional way to influence membrane permeability in artificial systems could be a new direction for such systems.



Figure 5. From the left to the right, together with the speakers of the second session Prof. Jun Li Hou, Prof. Phil Gale, the chair of the Session 2, Prof. Jefferey T. Davis, Dr. Yves-Marie Legrand, Prof. Harish Vashisth, the Vice Chair Prof. Manish Kumar and the Chair of the Faraday Discussion Dr. Mihail Barboiu and Prof. Robert J. Hickey.

Session 2 “Artificial (biomimetic) water channels”

The second session of the discussion focused on supramolecular approaches to channel like structures (Fig. 5). The early morning session focused on the formation of supramolecular tetrameric structures. Prof. Jefferey T. Davis from the University of Maryland discussed work with Songjun Xiao on the formation of hydrogels from 5-hydrazinoguanosine that self-assemble via the formation of G-quartets into hydrogels in the presence of 0.25 equivalents of KCl.¹⁰ The structural basis of the hydrogel - confirmed by PXRD, IR and CD spectroscopy - is the G₄ quartet which formed the basis of the extended 1-D ion channel assemblies. The hydrogel effectively binds anionic dyes such as naphthol blue black over cationic dyes. The hydrogel can also be functionalised by reaction with propionaldehyde in either the gas phase or in water solution via formation of covalent hydrazine linkages with the gel matrix. The next two presentations focused on the formation of selective water channels from imidazole-urea building blocks across lipid bilayer membranes. Dr. Yves-Marie Legrand presented work with Dr. Zhanhu Sun, Dr. Istvan Kocsis, Dr. Yuhao Li and Dr. Mihail Barboiu on structure activity relationships amongst this highly effective class of structurally simple yet highly selective water channel formers *via* the formation of an ‘I₄-quartet’.¹¹ A series of imidazole containing systems were prepared with varying chain lengths, different hydrogen bonding linking groups and, in some cases, double-headed systems containing two imidazoles. This study showed the importance of the urea moiety in organising the channel forming units in the lipid bilayer. Dr. Samuel Murail then presented work with Tudor Vasiliu, Dr. Andrei Neamtu, Dr. Mihail Barboiu, Dr. Fabio Sterpone and Dr. Marc Baaden on modelling of the I-quartet systems using MD simulations.¹² This work looked at the membrane stability of I-quartet aggregates taken from crystal patches, picosecond characterisation of water dynamics and self-aggregation simulations. The work provided two scenarios for the formation of water transport structures: ordered water wires *vs.* porous sponges. After coffee, the meeting reconvened for four presentations related to functionalised pillar[5]arenes as water channels. Prof. Jun-Li Hou presented work with Jin-Yu Chen, Wei-Wei Haoyang, Min Zhang, Gang Wu, and Prof. Zhan-Ting Li on the formation

of channels from pillar[5]arene functionalised with chains of Phe-Phe-Phe.¹³ Studies showed the incorporation of the compounds into model lipid bilayers which displayed channel-like conductance behaviour. Fluorescently labelled versions of the pillar[5]arenes were incorporated into mammalian cell membranes with a positively charged derivative inserting into the bilayer membrane of rat erythrocytes and human liver carcinoma cells HepG2. This compound was shown to reduce the cell viability of these cancer cells. The next presentation by Prof. Harish Vashisth with Daniel Ryan Barden who has looked at the parametrisation and atomistic simulations of biomimetic membranes e.g. block co-polymers with hydrophobic blocks of poly(butadiene) (PB) and hydrophilic blocks of poly(ethylene oxide) (PEO).¹⁴ They developed all-atom CHARMM force-field compatible parameter sets for various hydrophobic polymers. They also ran simulations of pillar[5]arene-based water channels embedded polymers and showed that the transport characteristics are similar to those observed in lipid bilayers. In the following presentation, Prof. Robert J. Hickey with Chao Lang, Yue-xiao Shen, Jacob A. LaNasa, Dan Ye, Woochul Song, Tawanda J. Zimudzi, Michael A. Hickner, Enique, D. Gomez, Esther W. Gomez, and Prof. Manish Kumar discussed creating cross-linked lamellar block copolymer supporting layers for biomimetic membranes.¹⁵ These workers demonstrated the feasibility of using triblock copolymer self-assembled lamellar films to form a matrix that mimics a lipid bilayer membrane. The final presentation of the morning session by Woochul Song with Dr. Yue-xiao Shen, Chao Lang, Prantik Saha, Iryna V. Zenyuk, Prof. Robert J. Hickey and Prof. Manish Kumar covered the incorporation of the pillar[5]arene water channels into PB-PEO block co-polymer sheets.¹⁶ The separation properties of these membranes were studied showing several unique selectivity trends.

Session 3 “The modelling and enhancement of water hydrodynamics”

The third session of the discussion focused on modelling water hydrodynamics in nanoscale pores, be it biological or artificial ones. Design principles for further enhancement of water permeation through such pores were considered (Fig. 6). Three complementary papers formed the basis of the discussion opened by Prof. Mark Sansom from Oxford University with a perspective on how to characterize the functional states of such pores, in particular, the ones found in ion channels, through the analysis of the wetting and dewetting behaviors combined with several channel metrics.¹⁷ Such an approach intrinsically requires taking into account the molecular scale dynamics of the pore region and is complementary to a purely geometric consideration of crystal structure pore dimensions. Further conceptual elements were added by Prof. Rob Coalson from University of Pittsburgh describing how complex biological channels such as Claudin can be examined through simple pore models capturing the essential features of the permeation machinery.¹⁸ A particular focus on the fine water substructures able to develop in these nano-environments was provided, and the effect of decorating the pore lining walls with charged groups was described. Selectivity of water *vs.* ion transport was touched on. Perspectives on using such pores for separation processes were given in the third paper by Prof. Manash Borthakur from the Indian Institute of Technology Guwahati.¹⁹ Using an oriented electric field, effects on a confined water-ethanol mixture in a carbon nanotube

environment were characterized, leading to the separation of both solvents under selected conditions.



Figure 6. Dr. Marc Baaden the chair of the Session 3, together with the speakers from the left to the right, Prof. Mark Sansom, Prof. Rob Coalson and Prof. Manash Borthakur.

Overall, the high potential offered by molecular simulations to advance our understanding of the molecular level water dynamics in artificial water channels has been brilliantly demonstrated by the constructive exchange with the presenting authors. Several of the stimulating discussions were taken over to the afternoon tea break and received extended attention. A second poster session followed, providing further material to discuss both molecular level but also macroscopic aspects of water transport, permeation and selectivity.

Session 4 “Applications to water transport systems”

The fourth session *Applications to water transport systems* (Fig. 7) began with a talk by Dr. Claus Helix-Nielsen from Aquaporin A/S and University of Copenhagen on conventional reverse osmosis (RO) membranes integrated with aquaporins (AQP).²⁰ The company represents the only commercially available water selective channel product, thus was central to the theme of the session. The paper was focussed on a review of large-scale membrane element design and a detailed description of the biochemical process to express and separate AQP in industrially relevant amounts. The overview presentation²¹ focussed on the larger picture of societal needs and the importance of engineering platforms that can be scaled compared to fragile lipid bilayers. This was a critical point since their product is based on conventional interfacial polymerization technology; with the addition of AQP infused vesicles as water channels at the membrane surface. The complication is that the RO membrane platform is itself a water selective membrane and the water flux value is sensitive to polymer processing. This makes it difficult to draw conclusions on the role of AQP channels. Shown in the presentation was an elegant control experiment to make membranes with wild and sequence modified AQP that rendered the channel blocked. The difference between open and closed AQP was roughly a factor of 2 allowing clear demonstration of flow through AQP

and relative amount of conventional RO membrane flux. Much of the discussion was focussed on ensuring water flux was through AQP and the complexity of RO membrane fabrication as a confounding variable. Estimates of AQP production of 270 gr/year per reactor were sufficient for $\sim 35,000$ m² of membrane in idealized monolayer geometry, which is a technically feasible arrangement for the field.



Figure 7. Prof. Bruce Hinds the chair of the Session 4, together with the speakers of the first part of the session 4 from the left to the right, Prof. Susana Nunes, Prof. Rob Coalson and Prof. Manash Borthakur.

This paper was followed by a highly scalable block-copolymer membrane with well-ordered lattices of nm-scale pore by the Nunes' group at KAUST.²² Processing conditions of solvent mixtures and evaporation rates were the primary tools of pore control. Discussion primarily focused on flow performance (permeance) and being a potential platform for integration of both protein channels and synthetic channels. Prof. Kazushi (Tokyo Inst. Technology) then followed with a detailed study of tri-block copolymers that form robust vesicles and channels formed via pi-stacking regions of designed bipyridines links.²³ Discussion focused on the structure of the channels and the types of selective transport observed.

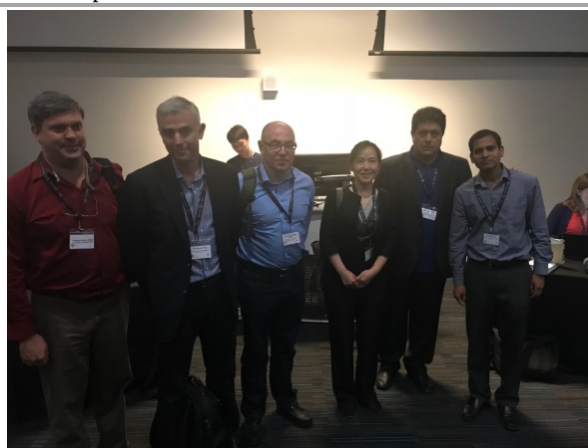


Figure 8. From the left to the right, Prof. Bruce Hinds the chair of the Session 4, together with the speakers of the second part of the session 4 Prof. Alexander Noy, Prof. Viatcheslav Freger, Prof.

For the second part of the session 4, Prof. Baoxia Mi (UC Berkeley) followed with an overview of 2-D graphene oxide GO membranes, where spacing between planes is controlled by the covalent molecular linkers.²⁴ Clear effects on water flux, ion rejection and molecular weight cut-off were shown. Discussion focused on the 2-D nanofluidics mechanisms in the defect rich GO system and potential linkers to improve performance to the level of being water selective channels. Prof. Gerhard Hummer from Max Planck Institute of Biophysics, Frankfurt am Main then described molecular dynamics (MD) simulations of small diameter CNTs spanning bilayers that complemented the following talk of Alexander Noy (LLNL) with experiments of short CNTs spanning lipid bilayer micelles.²⁵ In 2001, Prof. Hummer was the first to predict CNTs as a water selective channel with dramatic flow rates²⁶ (equivalent to AQPs) and work of Alexander Noy from Lawrence Livermore National Laboratory had recently shown CNT channels across vesicle walls to develop osmotic driven flow (with some salt selectivity) and similar flow rates.²⁷ The MD simulations showed a surprisingly complex interaction of CNTs with lipid bilayer but importantly, no water flow along the CNT-lipid interface and lipids did not block CNT channels.²⁵ Noy's experimental results²⁷ were of intensely ultra-sonically cut CNTs (~1 nm inner diameter, 10 nm length) spanning vesical lipid bilayers and showing predicted high flux/ion rejection as the vesicle shrunk in diameter from induced osmotic pressure and measured by established light scattering techniques. Discussion focussed on temperature studies showing the activation energy of water flowing across CNTs being at a relatively high value of 12 kcal/mol compared to natural AQP channels near 4 kcal/mol. An alternate mechanism of water flow through lipid/CNT interface or chaotic motion as opposed to through CNT core was suggested.²⁸ However MD simulations of Hummer did not show this to be a significant mechanism of water transport.²⁵ A confounding factor is the pre-exponential of Arrhenius equation (frequency of attempts varies widely in physical systems) and expected energy barrier of entering the hydrophobic CNT compared to the much shorter AQP constriction point but having unhindered flow after CNT entry. Also non-lipid bilayer CNT membranes from a number of groups see comparable flow rates though without ion rejection.



Figure 9 (top) Best poster presentation awarded to Shanlin Rao and Mr Gianni Klesse of the University of Oxford, United Kingdom (c) Members of the organising committee with the President of the Faraday Division of the Royal Society of Chemistry, Professor Claire Vallance MRSC during the Loving Cup Ceremony.

The final paper by Prof. Viatcheslav Freger from Technion expanded on the idea that 'too much success' in fast flow channels will generate 'concentration polarization' by building up too much salt to make very large effective osmotic pressures (effectively a system energy loss).²⁹ At a practical level *selectivity* is more important than flux (as concentration gradients build normal channels lose selectivity and stop working). However discussion focussed on the fact that AQP has near perfect selectivity and natural systems can have dense aquaporin arrays (as seen in animal eyes). It was agreed that water channels need selectivity as the primary figure of merit and there will be an inevitable trade off in terms of areal density (flow rate) vs. concentration polarization (salt build up on feed side). An order of magnitude reduction in RO membrane area by fast water channels is a reasonable goal for the community.

Poster session and social events

The social and networking periods involved most of the participants, who were actively engaged in discussing the 16 posters which have been presented as flash presentations by the presenters who received many questions on their posters, both by the judges and the delegates. The Faraday Discussions Poster Prize for the best poster was jointly awarded to Miss Shanlin Rao of the University of Oxford, United Kingdom, for her poster on "Hydrophobic gating: A systematic investigation of ion channel structures", and Mr Gianni Klesse of the University of Oxford, United Kingdom, for his poster on "CHAP: A new tool for functional

annotation of ion channel structures based on molecular dynamics simulation” (Figure 9 top).

The conference dinner allowed further exchanges and fruitful discussion in a relaxed environment. Most importantly, the participation in the famous Loving Cup Ceremony came as a special particular moment for most of the international delegates, however, everyone played the game until the end (Figure 9 bottom).

Concluding remarks lecture

The concluding remarks lecture on Artificial water channels: inspiration, progress and challenges was delivered by Prof. Bing Gong from University at Buffalo, USA (Fig. 10).



Figure 10. Professor Bing Gong from University of Buffalo after his concluding remarks lecture on Artificial water channels: inspiration, progress, and challenges together with the Vice Chair Prof. Manish Kumar and the Chair of the Faraday Discussion Dr. Mihail Barboiu.

He highlighted that biological channels revealed common structural details behind their extraordinary performances: the presence of a narrow pathway decorated with additional “check points” that provide both confinement and screening for the molecular and ionic species.³⁰ For water transport, the paramount importance of a sub-nm pore, preferably amphiphilic as shown by AQP_s,⁷ is also shown by CNT pores,²⁵⁻²⁷ graphene and graphene oxide²⁴ channels and more recently by synthetic molecular and self-assembling channels.^{1-4, 11-13} Fabricating biomimetic membranes with water channels incorporated into various 2D matrices, is still in its infancy but this strategy offers a very promising approach for developing the next-generation separation technologies. Important challenges include the precise control of pore length and the reduction of diameter down to the sub-nm range, the uniform or site-specific functionalization of the channel wall, the homeotropic alignment of water-transporting pores, and the fabrication of membranes with molecular-thickness. “Examining such artificial channels have yielded fascinating initial results, suggesting that artificial channels with properties paralleling those only observed with biological channels will no longer be a fantasy. Indeed, “There’s plenty of room at the bottom” for scientists to play with for years to come.”³⁰

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