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1       Micro- to nano-scale characterisation of polyamide  
2               structures of the SW30HR RO membrane using  
3               advanced electron microscopy and stain tracers

4  
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## 1 **Abstract**

2 The development of new reverse osmosis (RO) membranes with  
3 enhanced performance would benefit from a detailed knowledge of  
4 the membrane structures which participate in the filtration process.  
5 Here, we examined the hierarchical structures of the polyamide (PA)  
6 active layer of the SW30HR RO membrane. Scanning electron  
7 microscopy combined with focused ion beam milling (FIB-SEM) was  
8 used to obtain the 3-D reconstructions of membrane morphology with  
9 5 nm cross-sectional resolution (comparable with the resolution of  
10 low magnification TEM imaging in 2D) and 30 nm slice thickness.  
11 The complex folding of the PA layer was examined in 3 dimensions,  
12 enabling the quantification of key structural properties of the PA  
13 layer, including the local thickness, volume, surface area and their  
14 derivatives. The PA layer was found to exhibit a much higher and  
15 convoluted surface area than that estimated via atomic force  
16 microscopy (AFM). Cross-sectional scanning transmission electron  
17 microscopy (STEM) was used to observe the distribution of a tracer  
18 stain under various conditions. The behaviour of stain in dry and wet  
19 PA indicated that the permeation pathways have a dynamic nature and  
20 are activated by water. High resolution STEM imaging of the stained  
21 PA nano-films revealed the presence of <1nm pore-like structures  
22 with a size compatible with free volume estimations by positron  
23 annihilation lifetime spectroscopy (PALS). This study presents a  
24 comprehensive map of the active PA layer across different length  
25 scales (from micro- to sub-nanometer) and mechanistic insight into  
26 their role in the permeation process.

## 27 **Keywords:**

28 RO membrane, characterisation, FIB-SEM, STEM, permeation  
29  
30

## 1. Introduction

Reverse osmosis (RO) membrane filtration is frequently utilised in water purification and recycling for domestic, industrial and agricultural purposes [1,2]. Membranes designed for molecular separations in water usually comprise a “tight” separating layer of polyamide (PA) on a more open support layer of polysulfone (PSf). The PA and PSf layers are mounted on a woven fabric backing of polyethylene (PE) or polypropylene (PP) [3,4]. The topmost PA layer is thought to play the active role in the filtration process as a molecular sieve and an ion barrier [5]. Improved knowledge of the structure and chemistry of the PA layer is required to design membranes, which achieve higher performance in water desalination and purification [6]. Characterisation of the PA layer is particularly challenging due to its complex and interconnected ridge-valley structure, thinness of the active film, the sub-nanometre size of structures within the film, and beam sensitivity intrinsic to polymer materials in electron and ion beam microscopes.

The organisation of the PA layer at the meso-scale is often assessed by cross-sectional transmission electron microscopy (TEM) [7,8], isometric or top-down scanning electron microscopy (SEM) imaging [3,9–11] supported by topological information acquired via atomic force microscopy (AFM) [5,9,11,12]. The overall thickness of the PA layer ranges from 20 to 400 nm [12]. PA morphology is typically estimated by the maximum ridge-to-valley distance, average roughness and apparent surface area [5,13]. It is commonly accepted that water permeability increases with the apparent surface area of the PA layer [1].

The inner structures of the PA layer are usually characterised using electron microscopy (EM) imaging of membrane cross-sections [5]. The PA layer might be divided in two regions: a relatively flat and semi-porous base at the PA-PSf interface and a more open structure of

1 protuberances spreading from the base, giving rise to the well-known  
2 ridge-valley structure [14]. Recently, it has been suggested that the  
3 PA layer is formed of a continuous sub-10 nm, highly crumpled PA  
4 nano-film [15]. The crumpling of the thin nano-film results in the  
5 formation of multiple voids within the PA layer, especially in the  
6 basal region. Some studies suggest that these voids contribute to an  
7 increase in surface area (as the voids are open to the surface at one  
8 end [6,16]), which might be connected with increased flux [17–19];  
9 other studies suggested that the voids are discrete [19,20].

10 The molecular-scale mechanisms of particle/ion filtration are not fully  
11 understood and are typically rationalised in terms of classic pore-flow  
12 or solution-diffusion models [21,22]. Separation studies postulated  
13 that permeation is modulated by both continuous pores and the  
14 density of cross-linked polyamide [23,24]. Molecular dynamics (MD)  
15 simulations suggested that the filtration occurs through a network of  
16 sub-nanometre pores, which may play an active or passive role in the  
17 process [25,26].

18 “Porosity” at such small length scales likely corresponds to a  
19 modulation of density and free volume inherent to polymeric  
20 networks, rather than to actual pores in the macroscopic sense. In  
21 addition, segmental and cooperative polymer dynamics, under  
22 solvation and pressure conditions, might be expected to modulate  
23 solute transport [27]. Evidence of sub-nanometre pores within the PA  
24 layer has been obtained by positron annihilation lifetime spectroscopy  
25 (PALS) [5,22,28] and solution permeation rate calculations [29]. Until  
26 now, the only spatially resolved imaging methods used to study pores  
27 smaller than 10 nm were based on the tracking of nanoparticles [30].

28 Few attempts have been made to validate the permeation models  
29 (pore flow vs. solution diffusion) described above. Nanoparticles have  
30 been used as contrast agents in attempts to map classic pores and/or  
31 permeation pathways within the PA and PSf layers. To enhance the

1 visibility of these structures in transmission electron microscopy  
2 (TEM), membranes were infiltrated with nanoparticles such as Au or  
3 OsO<sub>2</sub> [14,30]. These studies used particles of known size to visualise  
4 the crumpled topography of the PA layer [14] and to measure the  
5 sizes of pores in the PA layer [30]. As the nanoparticles are often  
6 inhomogenously distributed within the membranes, it is difficult to  
7 observe the direct transport pathways or draw conclusions about the  
8 behaviour of membranes during the filtration process. Furthermore,  
9 nanoparticles of set size may not accurately reflect the size of voids  
10 within the membrane, due to complex transport mechanisms, the  
11 variation in the sizes of the pores or the dynamic nature of these  
12 pores.

13 In addition to nanoparticles, heavy metal stains such as osmium  
14 tetroxide, ruthenium tetroxide, uranyl acetate or lead citrate may be  
15 used to reveal the structural features of the PA layer. By contrast to  
16 nanoparticles, metal stains diffuse more homogeneously through the  
17 material due to their small size (initially ionic); selectively lodge in  
18 charged regions; additionally fix the material and could be delivered  
19 in both liquid and vapour phases. To date, staining has mainly been  
20 used to enhance contrast of membranes in TEM and map the  
21 distribution of functional groups in the PA layer [7,12,14]. As most of  
22 the TEM studies were conducted at relatively low magnifications, it is  
23 difficult to characterise any structural features smaller than 30 nm.

24 The question of how the micro- to nano-metre scale features and their  
25 morphology modulate the permeation process remain unanswered [6].  
26 Spatially resolved imaging techniques, such as SEM, TEM or AFM,  
27 do not provide a complete picture of the 3-dimensional (3-D)  
28 architecture of the PA layer and the impact of the structures within the  
29 PA on the filtration process at the micro- to nano-scale. At the sub-  
30 nanometre scale, little is known about the 3-D distribution of the  
31 pores, their chemistry and role in solute (water/ion) transport. PALS  
32 studies provide only statistical information about the bulk of the

1 membrane and computational models generally lack validation with  
2 spatially resolved experiments.

3 High resolution electron microscopy (EM) studies of RO membranes  
4 are so far limited. EM has mainly been used for relatively low  
5 magnification, bright-field TEM imaging showing cross-sections of  
6 commercial membranes [5,14] or SEM imaging of the PA surface  
7 [10,11]. However, EM methods are exceptionally well-suited to  
8 provide spatially resolved maps of the hierarchical structures of the  
9 polyamide layer. Advanced nanoanalytical techniques, available on  
10 modern scanning TEMs (STEMs), have a spatial resolution sufficient  
11 to observe structural features smaller than 1 nm.

12 The combination of tomographic EM methods allows 3-D  
13 visualisation of hierarchical membrane features from the micro- to  
14 nano-metre length scales. Yan et al. [16] highlighted the significance  
15 of large voids (10-50 nm in diameter) within the PA layer and the  
16 possible pathway in the BW30 membrane. Pacheco et al. [19] have  
17 recently examined void spaces (15 to 30 nm in diameter) within the  
18 PA layer of ESPA3 and SWC3 in 3-D and proposed a link between an  
19 increased volume of these voids with increased permeance of the  
20 membrane. A 2-D study by Lin et al. [20] estimated that these voids  
21 may constitute up to 30% of the PA layer volume. The focus of our  
22 work is therefore to comprehensively examine and precisely quantify  
23 the structures within the PA layer envelope at the micro-scale, and  
24 examine structures within the PA nano-film at the nano- to sub-nano-  
25 scale with unprecedented spatial resolution.

26 We combine a range of EM techniques with heavy metal staining  
27 methods to visualise the hierarchical structure of the PA layer of the  
28 commercially available SW30HR (Dow Filmtec) membrane, selected  
29 for its common industrial use in high rejection water desalination  
30 [31].

1 Focused ion beam scanning electron microscopy (FIB-SEM) was  
2 used to reconstruct the features of polyamide in 3-D with high spatial  
3 resolution. Staining was used not only for contrast enhancement, but  
4 also for elucidation of the PA structure, specifically for mapping the  
5 nano-pores within the PA nano-film using high annular dark field  
6 scanning transmission electron microscopy (HAADF-STEM; atomic  
7 number,  $Z^2$  contrast imaging). Moreover, by varying the staining  
8 methods, potential permeation mechanisms in the polyamide film  
9 could be proposed.

## 10 **2. Materials and methods**

### 11 **2.1. Sample preparation for electron microscopy**

12 SW30HR (Dow Chemical Company, USA) flat sheet membranes  
13 were stained with  $\text{RuO}_4$ . In general, staining improves the overall  
14 contrast for electron microscopy (EM) imaging, stabilises the polymer  
15 under the electron beam (by crosslinking oxidised moieties, such as  
16 amide and carbonyl groups) and highlights the distribution of  
17 functional groups. The varying behaviour of stain tracers in different  
18 staining conditions may also reveal the permeation pathways.

19

20  $1 \times 1 \text{ cm}^2$  membrane coupons cut from a larger sheet were stained  
21 using standard (as received) stabilized 0.5 wt.% aqueous solutions of  
22  $\text{RuO}_4$  (Acros Organics, Geel, Belgium). Four different staining  
23 methods were used:

- 24 I. A membrane coupon was fully immersed in liquid stain for 5  
25 min and immersed in 10ml of water for 30 min;
- 26 II. The top surface of membrane coupon was exposed to the stain  
27 vapours for 1 min;
- 28 III. A drop of liquid stain was placed on the top surface of  
29 membrane coupon for 1 min;



1 IV. A drop of pure water was placed on the top of the  
2 membrane coupon for 10 min, and then the top surface of the  
3 coupon was exposed to the stain vapours for 1 min.

4 Method I was used to enhance contrast for FIB-SEM imaging (SI  
5 Figure 1) to ensure that the polymer was stained throughout. Methods  
6 II, III and IV were used to examine permeation pathways within the  
7 PA layer by STEM. To ensure the reproducibility of the results, 3  
8 samples for each of the staining conditions were prepared. The  
9 combination of dry (method II) and wet techniques was used to probe  
10 the transport mechanisms of ions and to highlight the role of water in  
11 the permeation process. Staining was performed under ambient  
12 conditions. Longer staining times (5 and 10 min) were also tested for  
13 methods II-IV.

14 To prepare samples for EM, the stained samples were washed 3 times  
15 with water for 5 min each to remove excess stain. Next, to dehydrate  
16 the samples, they were immersed in 50%, 70%, 90% and 100%  
17 aqueous solutions of ethanol (30 min each). Samples were embedded  
18 in LR White Hard resin (London Resin Company, Reading, UK),  
19 which was found to give the best contrast from the PA polymer.  
20 Samples were immersed successively in 1:1 and 3:1 resin:ethanol  
21 solutions for 12h. For the next 4 days, samples were immersed in pure  
22 resin to ensure full infiltration. The resin was changed every 24h. On  
23 the last day samples in resin were degassed under vacuum to ensure  
24 sample-resin binding and resin hardening. The final resin batch was  
25 mixed with a dedicated LR White accelerator (benzoyl peroxide) to  
26 enhance the crosslinking of the resin at room temperature. Pristine  
27 (non-stained) samples were embedded using the same protocol.

## 28 **2.2. Focused ion beam SEM (FIB-SEM)**

29 For FIB-SEM milling, a flat surface of the resin embedded sample  
30 stained, using method I, was exposed using a PowerTome  
31 ultramicrotome (Boeckeler Instruments, Tucson, USA) with a

1 diamond knife (DiATOME, Hatfield, USA). Next, a 1 mm thick slice  
2 was cut and mounted on an aluminium stub using silver paint. Prior to  
3 SEM imaging, the sample was coated with a 20 nm layer of  
4 chromium to reduce charging.

5 FIB-SEM series were taken on an Auriga microscope (Carl Zeiss  
6 Microscopy, Jena, Germany) fitted with a gallium ion source. The  
7 FIB milling conditions were set to 30 kV and with a beam current of 1  
8 nA, which results in a probe size of ~10nm.

9 Samples were milled from the side (SI Figure 2). To minimise FIB  
10 damage (e.g., ion implantation and curtaining) during alignments, the  
11 top, 1 $\mu$ m-thick part of sample was used as a sacrificial layer. Only the  
12 region below the sacrificial layer was imaged by SEM (SI Figure 2).

13 SEM imaging was performed at 1.49 kV with an energy selective  
14 backscattered electron detector. In these conditions, the size of SEM  
15 probe is ~2nm. Pixel averaging collection was used to reduce noise.  
16 In total, 357 images of membrane cross-sections were acquired, with  
17 a step size of 30 nm between successive images. As SEM images  
18 were taken at an angle (SI Figure 2), the images had to be adjusted to  
19 account for this resulting in non-uniform pixels (the pixel size in y  
20 direction was multiplied by 1.24).

21 Avizo software (FEI, Hillsboro, USA) was used for pre-processing of  
22 FIB-SEM data and reconstruction of virtual volumes. FIB-SEM  
23 slices were denoised using a Gaussian filter and realigned using an  
24 automatic least-squares function. Further information on thresholding  
25 and reconstruction can be found in SI (SI Figure 3). The reconstructed  
26 voxel size was 3.7x4.6x30 nm<sup>3</sup>.

### 27 **2.3. Estimation of structural properties**

28 To minimise errors in the calculation of membrane parameters arising  
29 from non-uniform voxels [32], volumes reconstructed from FIB-SEM  
30 data were resliced in ImageJ [33] to obtain uniform voxels of 5x5x5

1 nm<sup>3</sup>. To accurately assess the outer volume of the PA layer envelope  
2 and the impact of the discrete objects within the envelope on the  
3 structural parameters, the volume was purified (discrete voids and  
4 particles were removed from the volume – SI Figure 4) in BoneJ [34].  
5 The PA layer is very heterogeneous; to address the natural variability  
6 of the membrane, 2.5x2.5 μm<sup>2</sup> regions were examined.

7 BoneJ was used to determine the total surface area (SA), volume (V)  
8 and local thickness [35] distribution of the PA layer envelope. The  
9 local thickness at any given point (voxel) of a 3-D structure is defined  
10 as the diameter of the largest sphere that fits inside the 3-D object and  
11 contains the point [35]. In other words, the individual local thickness  
12 measurement represents the local diameter of the structure. A graphic  
13 explanation of the local thickness measurement is given in SI Figure  
14 5. The rudimentary reactivity was estimated as surface-to-volume  
15 (SA/V) ratio. Front and rear surface areas were calculated from the  
16 total surface area of front and rear volumes by subtracting the side  
17 and division areas (SI Figure 4). Relative area ratios were calculated  
18 by dividing the actual surface area by the area of the underlying  
19 rectangle.

#### 20 **2.4. Atomic force microscopy (AFM)**

21 For comparison with the surface structure generated from FIB-SEM,  
22 AFM imaging was used. The front surface of the PA layer was  
23 imaged on the original SW30HR membrane taped to an AFM sample  
24 holder with the PA facing upwards. To image the rear surface of the  
25 PA layer, free-standing PA layers were prepared and placed on a glass  
26 cover slip. The freestanding membranes were obtained as follows.

27 The polyethylene (PE) backing was carefully peeled off of the  
28 SW30HR membrane with a scalpel blade. The remaining two-layered  
29 membrane was pressed onto a clean glass cover slip such that the PA  
30 side sticks to the glass substrate. A drop of deionized water was added  
31 to hydrate and soften the membrane. A few drops of

1 dimethylformamide (DMF) were carefully placed onto the PSf facing  
2 upwards. As the PSf dissolved in the solvent, the glass slide was tilted  
3 to allow the solvent with the dissolved PSf to be drained. The process  
4 was repeated several times until the PSf was completely removed.  
5 The isolated PA thin layer appeared optically transparent.

6 Samples were imaged in an Innova AFM (Bruker, USA) in tapping  
7 mode fitted with a phosphorus (n) doped silicon cantilever ( $k = 20\text{-}80$   
8  $\text{N/m}$ , tip nominal diameter  $<10\text{ nm}$ ). AFM images were acquired at  
9 512 samples/line and a scanning rate of 0.2 Hz, under ambient  
10 conditions.

## 11 **2.5. Scanning transmission electron microscopy (STEM)**

12 For TEM imaging, thin ( $\sim 70\text{ nm}$ ) sections of samples were cut with  
13 the ultramicrotome (Boeckeler Instruments, Tucson, USA) and  
14 transferred onto 300-mesh copper grids with a lacey carbon film  
15 support (TAAB Laboratories Equipment, Aldermaston, UK).

16 STEM imaging was performed on a  $C_s$  (image-)corrected, FEI Titan  
17 80-300 microscope operated at 80 and 300kV. A 60 mm camera  
18 length was used giving an annular dark field (ADF)-STEM collection  
19 semi-angle of 26-145 mrad and high angle (HA)ADF-STEM  
20 collection semi-angle of 129-575 mrad. Before imaging, samples  
21 were beam showered (exposed to an uncondensed broad electron  
22 beam) in the TEM mode to stabilise the sections by equalizing the  
23 charge distribution across the section and reduce hydrocarbon  
24 contamination [36]. The experimental dose was closely monitored and  
25 did not exceed  $1250\text{ e/nm}^2$ .

26 High resolution STEM imaging was performed on SuperSTEM2 -  
27 Nion UltraSTEM 100 microscope at 100kV. The HAADF collection  
28 semi-angle range is 85-190 mrad and the probe size is 0.1 nm. Prior to  
29 imaging, samples were baked for 12h in a vacuum oven ( $10^{-6}\text{ Torr}$ )  
30 with a rising temperature gradient (from room temperature to  $130^\circ\text{C}$ )  
31 to reduce hydrocarbon contamination.

## 2.6. Quantification of the <1 nm structures

High resolution images were denoised using a Gaussian filter, contrast-enhanced and thresholded using magic wand selection. We aimed to highlight only structures that showed local maxima. In some instances we used a water-shedding algorithm to separate larger clusters of stain precipitates. The surface areas of the reconstructed precipitates were calculated using ImageJ (SI Figure 6). The diameters of the pores were estimated as the diameter of circles of the same area. All spots of diameter smaller than 0.2 nm were filtered out (the approximate size of Ru atoms  $\approx$  0.15 nm).

## 3. Results

### 3.1. 3-D structure of the polyamide layer

To reconstruct and quantify features of the PA layer morphology, cross-beam FIB-SEM tomography was used. We are able to visualise features larger than 15 nm in the PA layer in 3-D. The 3-D reconstruction in Figure 1 and SI Movie 1 shows the front (Figure 1A) and rear (Figure 1B) surface, and cross-sections (Figure 1 C, D) of the PA envelope. The envelope represents a volume containing the entire PA layer with the discrete, inner voids excluded. Orthoslices taken through the 3-D reconstructions show that protuberances, which are often referred to as ridges [5], are in fact leaf-like (bridge-like) sheets overhanging large regions of the PA layer (Figure 1 C, D - arrowheads). These regions are not fully enclosed and large empty spaces directly underneath the ridges are open to the front surface. There were also smaller, discrete voids observed within the PA envelope (Figure 2 and SI Movie 2). These discrete voids have an average local thickness of  $21\pm 7$  nm (367 voids for the minimal threshold) and  $17\pm 5$  nm (61 voids for the maximal threshold).

Local thickness mapping [34] was also performed on the bulk features of the PA layer (Figure 3A, B and C). The local thickness describes dimensions of all PA layer features: from a single sheet ( $\sim$ 10 nm) to

1 multiple convoluted and compacted sheets of PA nano-film (~260  
 2 nm). The latter structures appear as non-porous (Figure 3 B, E – white  
 3 arrowheads), but may possess void spaces, which are below the  
 4 resolution of the FIB-SEM technique or remain unresolved due to  
 5 poor contrast.

6 The ridge-like features (Figure 1A and C) on the front side of the  
 7 membrane were found to be <70 nm thick, whereas the features on the  
 8 rear side of the PA layer were much thicker (up to 260 nm), as AFM  
 9 cannot measure overhangs, steep walls and other convoluted  
 10 structures of the PA topography. For comparison, AFM maps were  
 11 converted into 3-D models and local thickness maps were calculated  
 12 (Figure 3D, E and F). The measured root-mean square roughness was  
 13 75 nm and the peak-to-peak height was 496 nm for the front surface,  
 14 and 15nm and 110nm, respectively, for the back surface. In the AFM  
 15 reconstruction, almost all of the membrane volume appeared thick  
 16 (bright), because this method only maps the apparent topography. In  
 17 Table 1, the structural properties of the PA layer in the SW30HR  
 18 membrane are given.

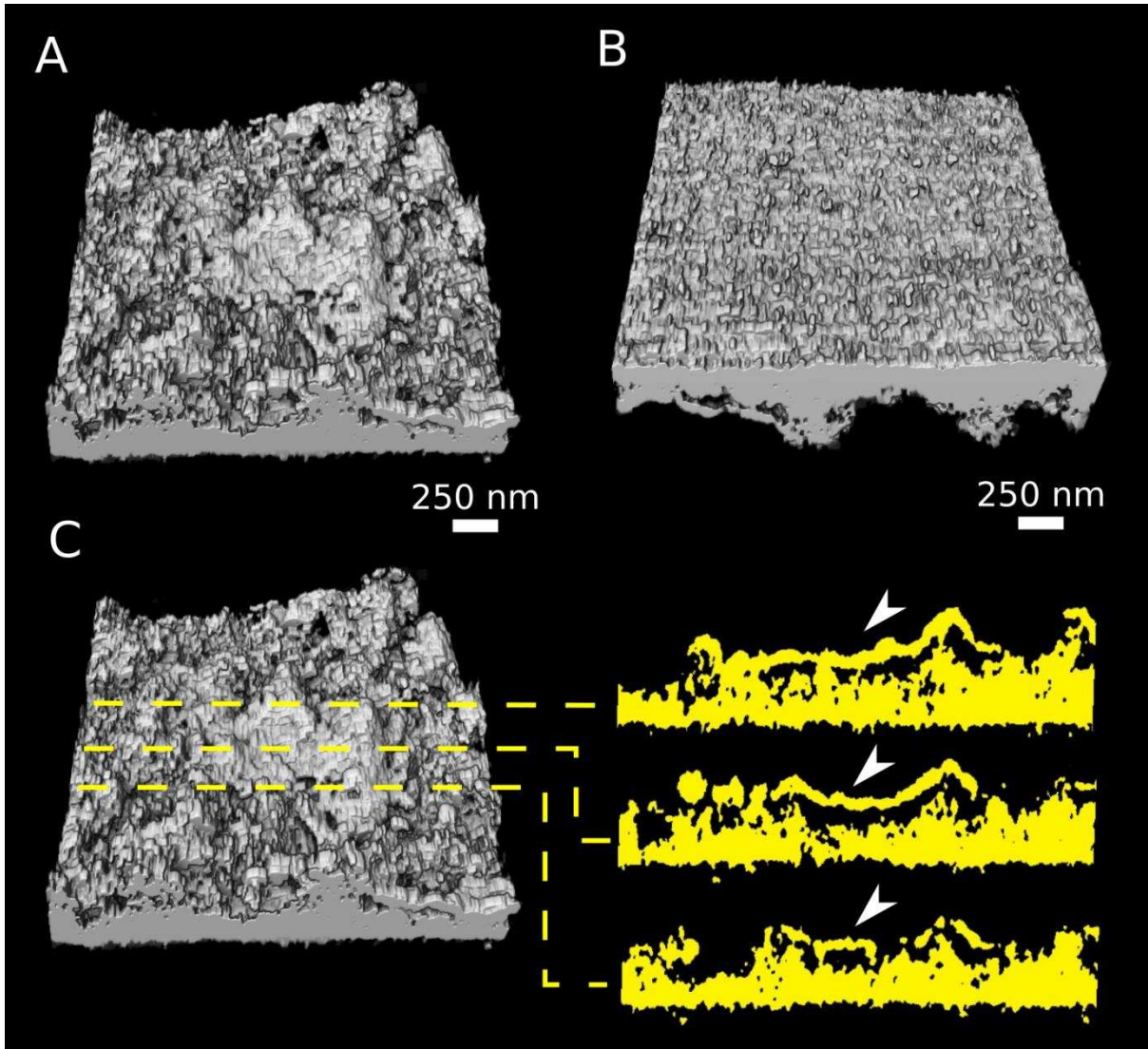
19 **Table 1 Structural properties of the PA layer of a SW30HR**  
 20 **membrane (for 2.5x2.5  $\mu\text{m}^2$  regions).**

	Mean local thickness (nm)	Maximal local thickness (nm)	Reactivity SA/V ( $\text{nm}^{-1}$ )	Relative area ratio - front	Relative area ratio - rear
<b>PA envelope (MIN*)</b>	96±42	206	0.037	6.71	1.87
<b>PA envelope (MIN Purified)</b>	125±61	260	0.033	5.98	1.67
<b>PA envelope</b>	179±92	344	0.028	4.9	3.55

<b>(MAX)</b>					
<b>PA envelope (MAX Purified)</b>	183±90	344	0.025	4.66	2.64
<b>AFM</b>	284±71	443	0.012	1.71	1.3**
<b>AFM Purified</b>	285±70	443	0.012	1.64	1.3**

1 \* MIN – minimal threshold, MAX – maximal threshold, Purified –  
2 discrete voids removed

3 \*\* AFM front and rear surface images were collected from different  
4 regions.

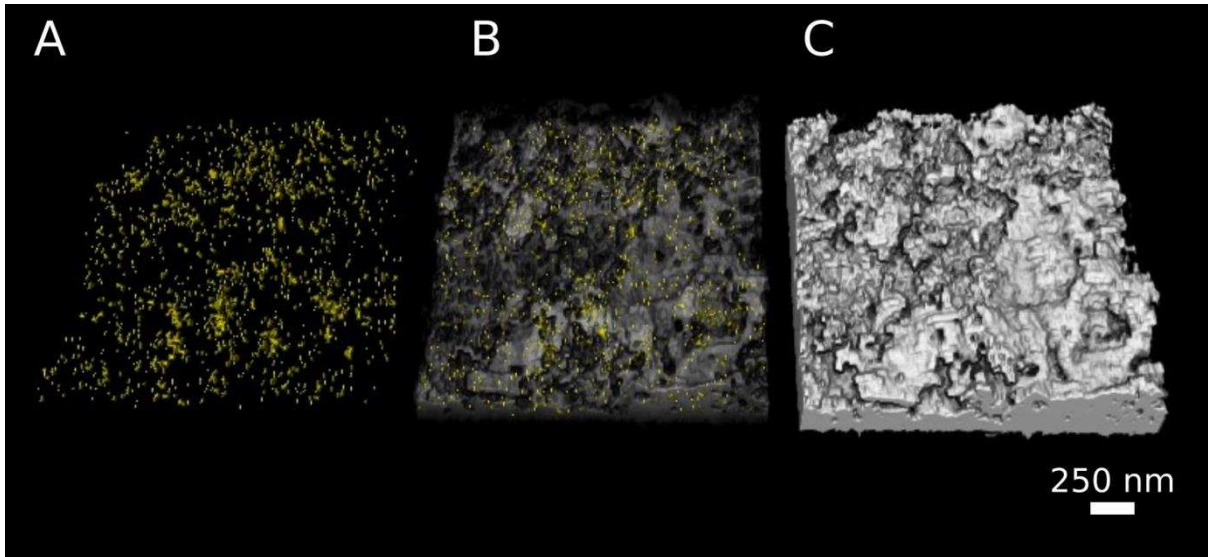


1

2 **Figure 1 A FIB-SEM 3-D reconstruction of the PA layer envelope**  
3 **showing: (A) the front and (B) rear surfaces of the PA and (C)**  
4 **cross-sections through the ridges showing that they are hollow**  
5 **(white arrowheads). All voids seen in (C) are open to the surface.**

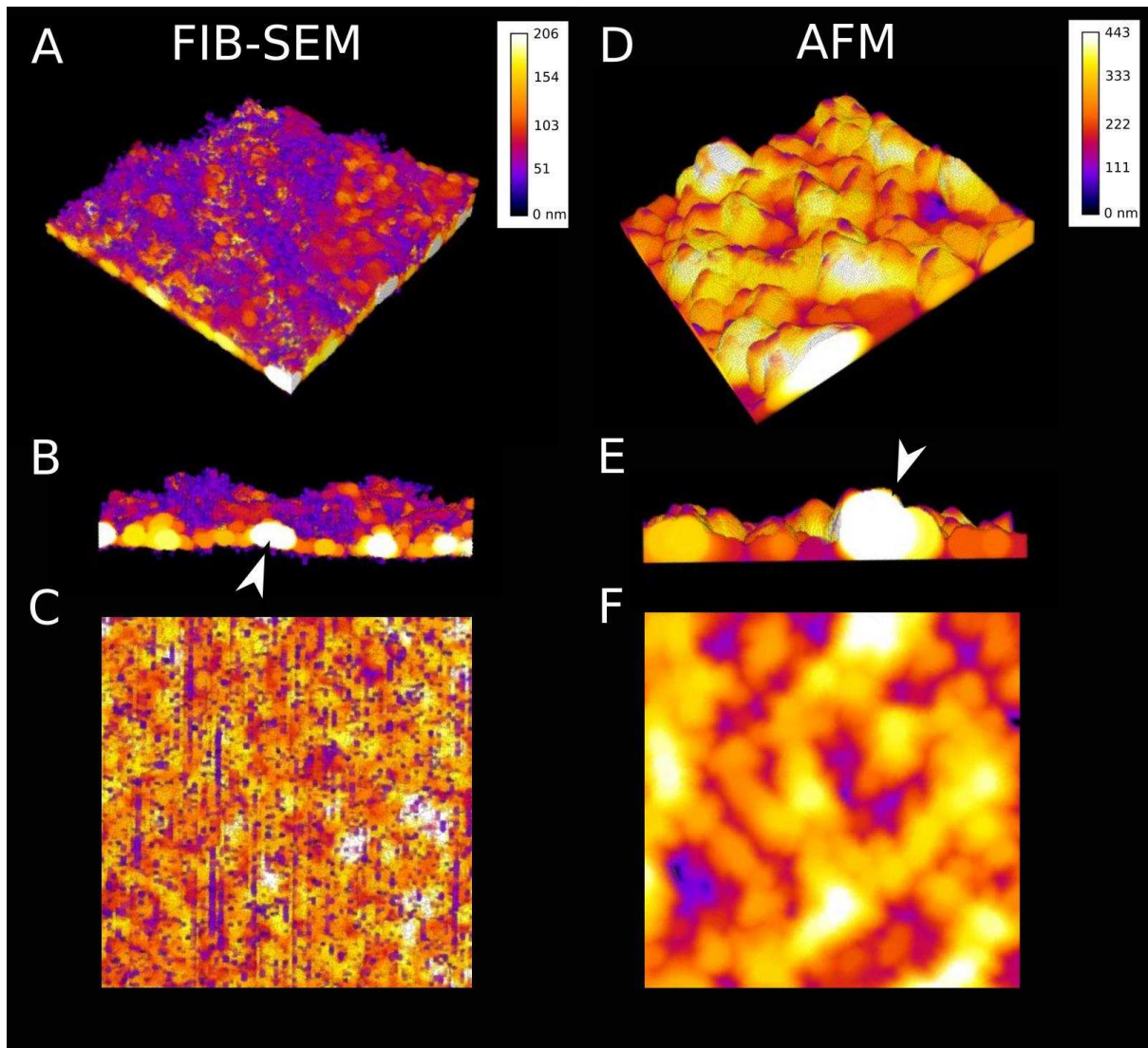
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1

2 **Figure 2 A FIB-SEM 3-D reconstructions of discrete voids**  
3 **(yellow) and the PA envelope (grey). (A) Discrete voids extracted**  
4 **from the PA reconstruction through a purification routine. (B)**  
5 **The same voids (A) overlaid with a semi-transparent PA**  
6 **envelope. (C) A fully opaque reconstruction of the PA envelope in**  
7 **the given region.**



1

2 **Figure 3 A local thickness map of the PA envelope: (A) an**  
 3 **isometric, (B) side and (C) a rear view from a FIB-SEM**  
 4 **reconstruction and (D) an isometric, (E) side and (F) a rear view**  
 5 **from an AFM reconstruction. Thin regions are dark (red to**  
 6 **violet), while thick regions are bright (white to yellow; the**  
 7 **thickest structures for each reconstruction are indicated by white**  
 8 **arrows). Each view shows a  $2.5 \times 2.5 \mu\text{m}^2$  region.**

### 9 **3.2. Inner structure of PA layer**

10 Staining experiments were used to reveal the inner structures of the  
 11 PA layer, seeking to resolve possible permeation pathways, and thus,  
 12 transport mechanisms through the PA nano-film.

1 In Figure 4, the effects of vapour staining on a dry membrane (method  
2 II) are shown. As the vapour stain reached the top surface of the  
3 membrane, stain propagation was evidently hindered at the PA-air  
4 boundary. The stain did not penetrate readily into the PA nano-film  
5 and instead precipitated forming a bright crust along the top interface  
6 (Figure 4 A, B). Bright spots within the PA nano-film were also  
7 observed (Figure 4C), suggesting that some stain precipitated within  
8 the pores of the PA. Many of the voids observed at the rear side of the  
9 PA layer (i.e., inside the basal region next to the polysulfone support)  
10 were not accessed by the stain. Stain precipitates were observed in  
11 only a few of these voids (Figure 4A, arrowheads), which are most  
12 likely open to the top surface. Occasionally, stain precipitates  
13 aggregated at the entrance to a seemingly open void (Figure 4D and  
14 SI Movie 3). In general, the propagation of stain through a dry PA  
15 layer was significantly impeded.

16 The liquid staining technique (method III) sought to visualise the  
17 transport of water in the PA layer. As the stain is in an aqueous  
18 solution, the distribution of tracer should, in principle, reflect the  
19 fraction accessible to water molecules within the PA layer. In Figure  
20 5, the effects of liquid staining on the front side of the membrane are  
21 shown, in clear contrast to the dry images above. When the liquid  
22 stain was applied on the top surface, the stain propagated through the  
23 PA layer and into the PSf support. Voids on the rear side of the PA  
24 layer (towards the PA/PSf interface) and very complex structures  
25 within the PA were penetrated by the stain (Figure 5 A, C). Staining  
26 highlighted the presence of effective “channels” within the PA layer  
27 (Figure 5 B, D), which were either filled with a dense precipitate  
28 (black arrowheads) or were empty (white arrowheads). Such  
29 ”channels” were also observed in non-stained samples (Figure 6), but  
30 staining significantly enhanced their visibility. These structures have  
31 significantly weaker contrast in the pristine (non-stained) PA, which

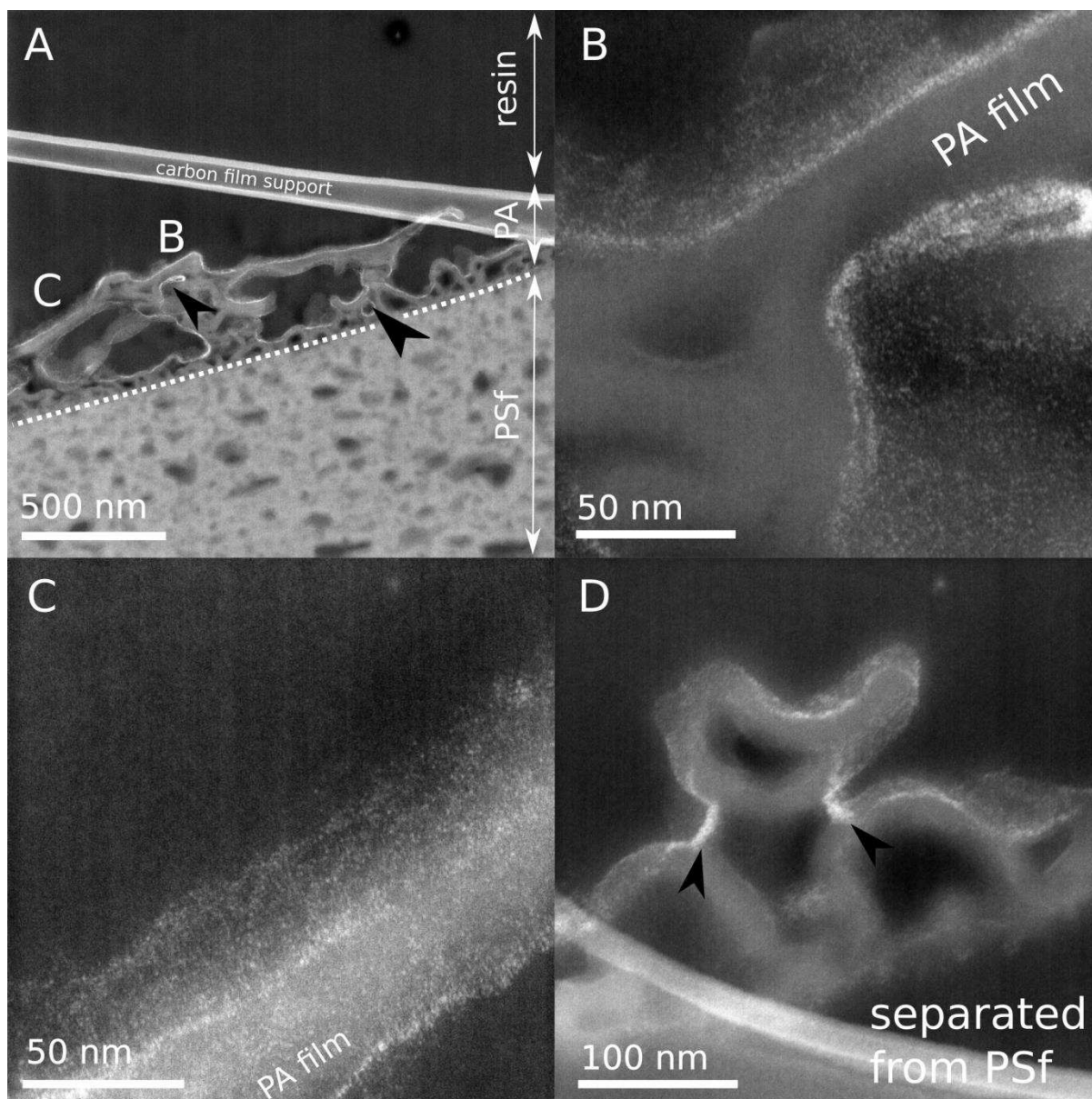
1 suggests that these are hollow regions formed by tight folding of the  
2 PA film.

3 The staining patterns clearly differed between the dry and wet  
4 experiments presented above. Therefore, membrane solvation and  
5 swelling in water and must play a significant role in activating the  
6 permeation pathways within the PA. To test this hypothesis, the  
7 vapour staining technique on a wet membrane (method IV) was used  
8 to further investigate the role of water and access of transport  
9 pathways in the membrane. In Figure 7, the effects of vapour staining  
10 on a wetted membrane were shown. In comparison to the dry  
11 membrane (Figure 4) the stain started to reach enclosed regions of the  
12 PA layer (Figure 7A), but did not penetrate the PA as thoroughly as  
13 under wet conditions (Figure 5). Smaller precipitates within the PA  
14 nano-film were often observed and occasionally, a stain propagation  
15 front could be seen (Figure 7B).

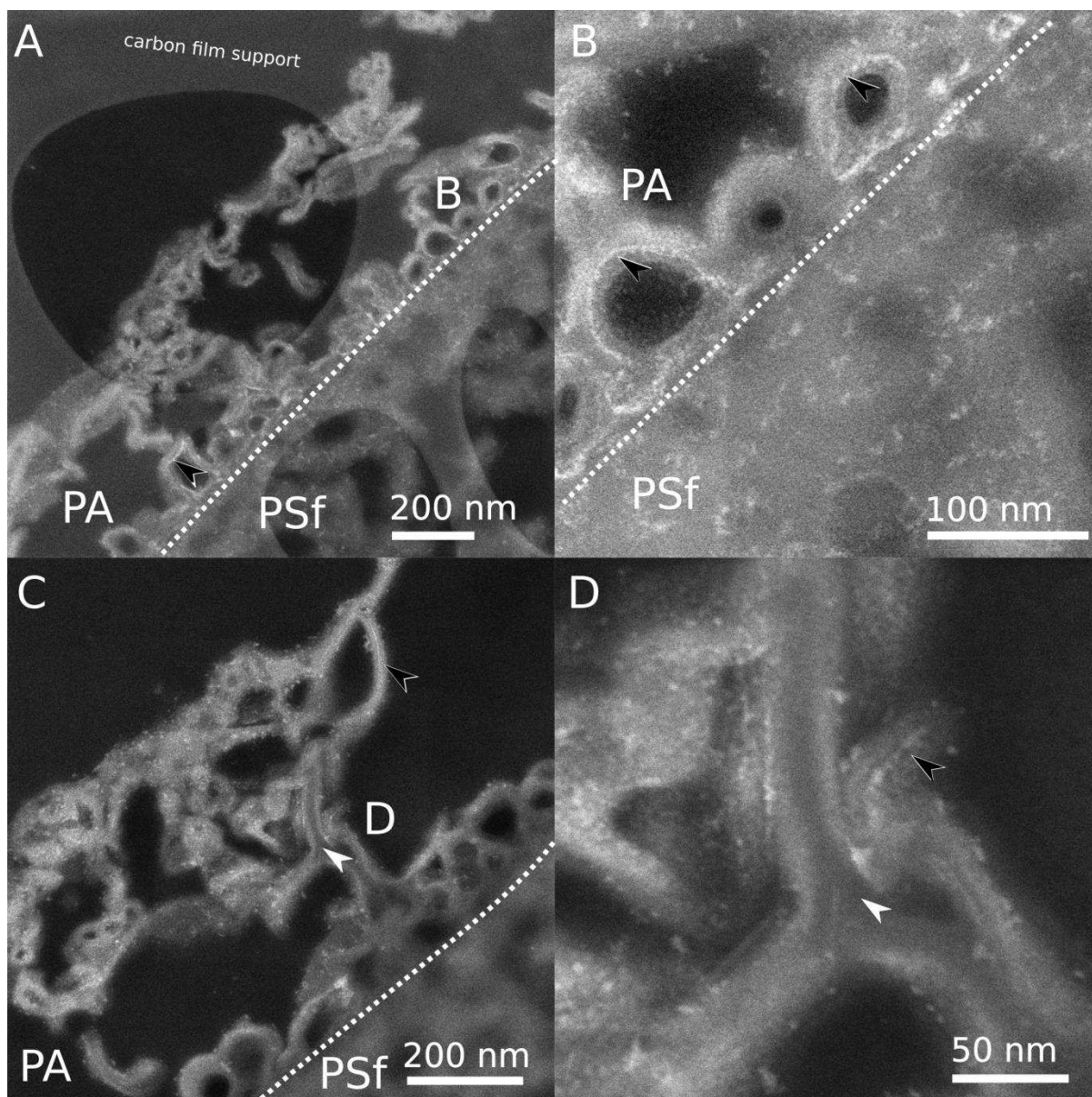
16 For longer staining times (5 and 10 min), precipitates outside the PA  
17 layer dominated the contrast (SI Figure 7).

18

19

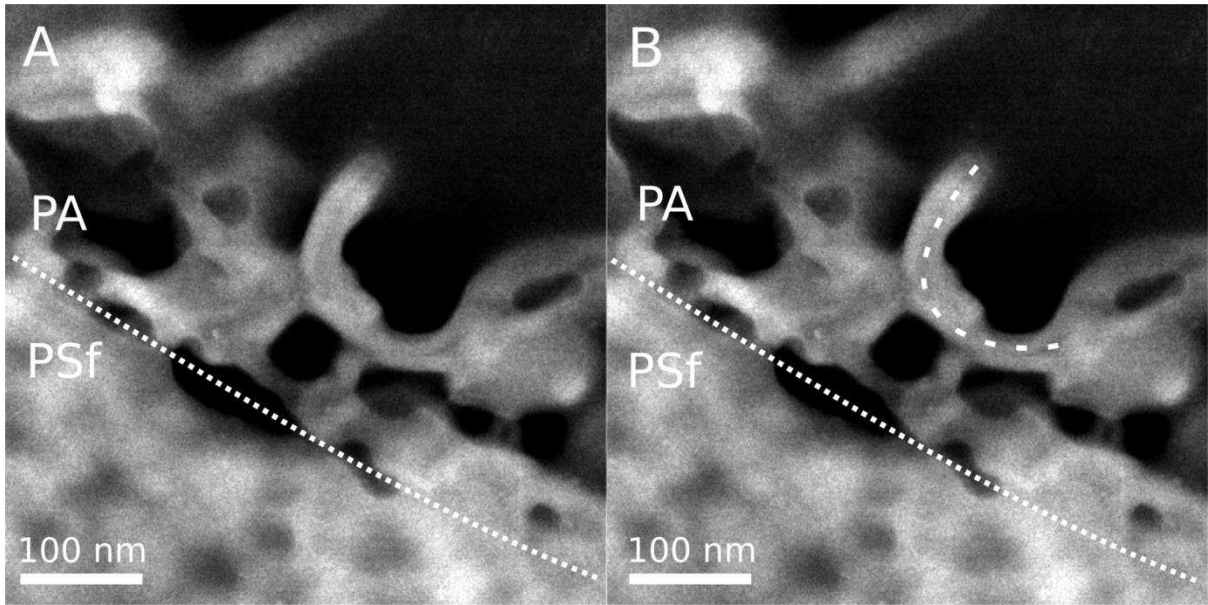


1  
2 **Figure 4 HAADF-STEM images of a dry SW30HR membrane,**  
3 **which was vapour stained on the front side with RuO<sub>4</sub> for 1 min.**  
4 **(A) Stain precipitates formed on the surface of the PA layer and**  
5 **within few voids (black arrowheads) within the layer. The dotted**  
6 **line represents the PA/PSf interface. (B) Higher magnification**  
7 **image of the region seen in (A) showing stain on the outside of the**  
8 **nano-film. (C) Higher magnification image of the region seen in**  
9 **(A) showing stain precipitates within the PA nano-film. (D) Stain**  
10 **precipitates aggregating (black arrowheads) in the entrance to a**  
11 **seemingly open void.**



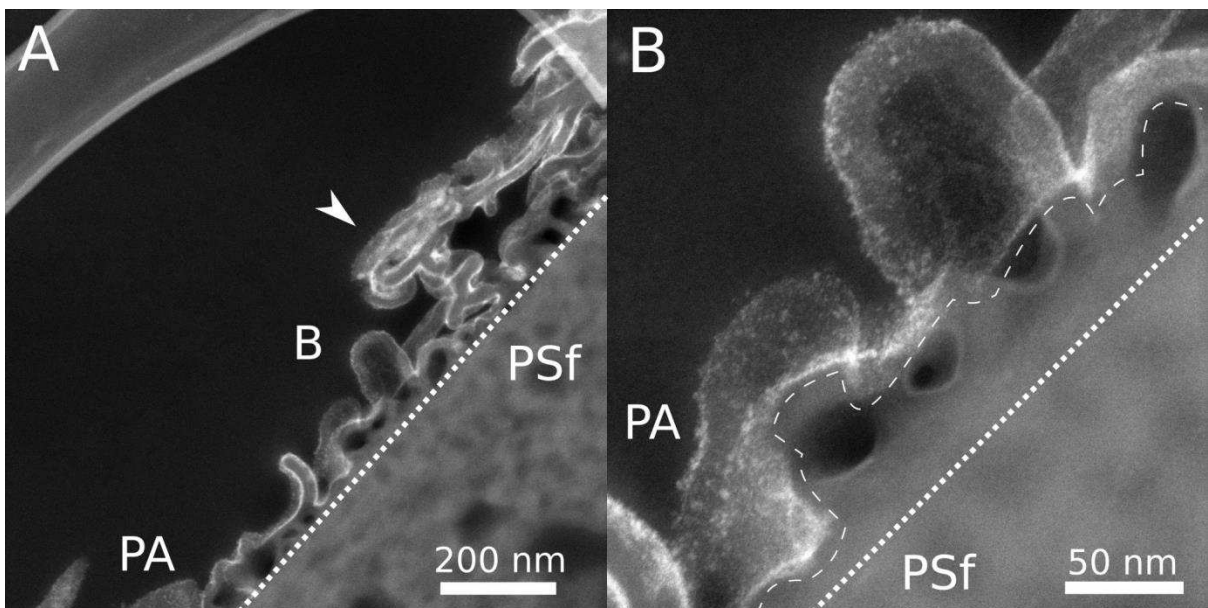
1

2 **Figure 5 HAADF-STEM images of a SW30HR membrane stained**  
 3 **from the front side with RuO<sub>4</sub> in aqueous solution for 1 min. (A)**  
 4 **Stain precipitates were present in the whole volume of the PA**  
 5 **layer. (B) Higher magnification image of the region seen in (A)**  
 6 **showing dense precipitates (black arrowheads) formed within**  
 7 **“channels” of the basal PA layer. (C) Selective staining showing**  
 8 **empty (white arrowheads) and occupied (black arrowheads)**  
 9 **“channels” within the PA. (D) Higher magnification image of**  
 10 **empty (white arrowheads) and filled (black arrowheads)**  
 11 **channels seen in (C). The dotted lines in (A), (B) and (C)**  
 12 **represent the PA/PSf interface.**



1

2 **Figure 6 HAADF-STEM images of a non-stained SW30HR**  
 3 **membrane. (A) An acquisition showing empty channels within the**  
 4 **PA layer. (B) A respective image with highlighted “channels” - ,**  
 5 **possibly representing collapsed voids. The dotted lines in (A) and**  
 6 **(B) represent the PA/PSf interface.**



7

8 **Figure 7 HAADF-STEM images of a wetted SW30HR membrane,**  
 9 **which was vapour stained on the front side with RuO<sub>4</sub> for 1 min.**  
 10 **(A) Stain precipitates formed on the surface and within**  
 11 **convoluted regions (white arrowhead) of the PA layer. (B) Higher**  
 12 **magnification image of the region seen in (A) showing stain within**

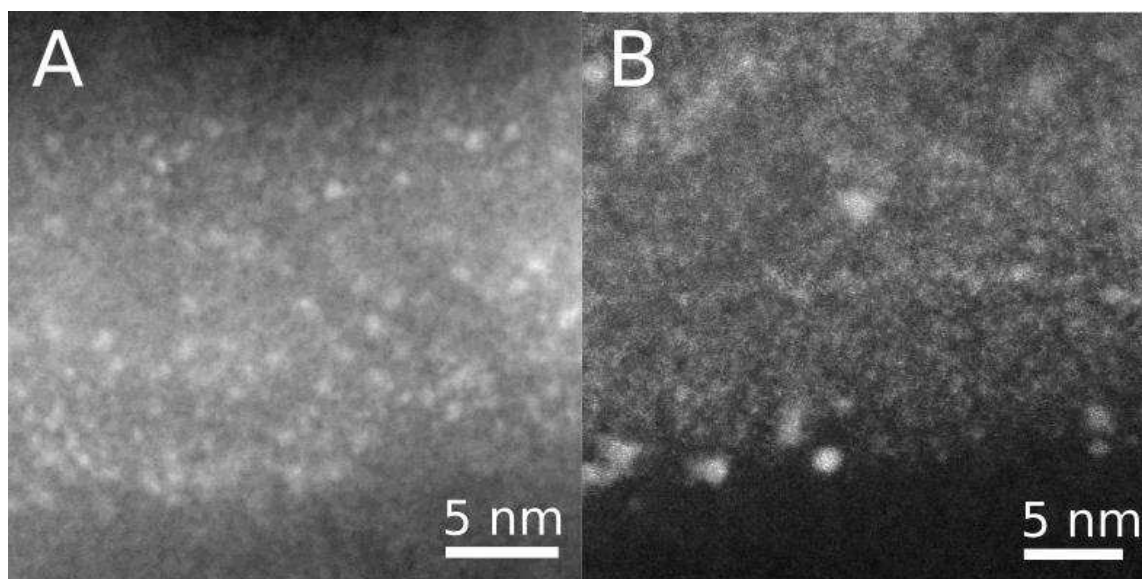
1 **the nano-film and a stain propagation front (dashed line). The**  
2 **dotted lines in (A) and (B) represent the PA/PSf interface.**

### 3 4 **3.3. Porosity of the polyamide nano-film**

5 Until now the presence and dimensions of sub-nanometre pores  
6 within the PA nano-film have been estimated via indirect techniques,  
7 such as PALS [5]. The observation of <1 nm pores has been  
8 significantly hindered by intrinsic low contrast of polymer material  
9 and shortcomings of imaging techniques. With the combination of  
10 high spatially resolved EM imaging and staining methods described  
11 above, <1 nm pores can be visualised. The stain highlights circular  
12 regions within the PA nano-film (Figure 8). This contrast arises from  
13 stain precipitates lodged within the nano-film.

14 For high resolution STEM imaging, regions where precipitates had  
15 formed within the PA nano-film (stained with method II and III) were  
16 selected. Only sections with the stain evenly distributed across the  
17 cross-section were examined to ensure that the observed precipitates  
18 were inside the PA nano-film and not on its surface. The micrographs  
19 show the presence of precipitates within the PA nano-film (Figure 8).  
20 Both images show a fragment of a PA nodule at high magnification  
21 (SI Figure 8). The average diameter of precipitates formed within the  
22 nano-film is <1 nm ( $0.7\pm 0.4$  nm for vapour stained;  $0.6\pm 0.4$  nm for  
23 liquid stained;  $n=250$ ).





1

2 **Figure 8 HAADF-STEM images of stain precipitates in the PA**  
3 **nano-films of the SW30HR membrane exposed to (A) the vapour**  
4 **and (B) liquid stain for 1 min (method II and III). Both images**  
5 **show cross-sections through the protuberances at high**  
6 **magnification (SI Figure 8).**

#### 7 **4. Discussion**

8 Staining combined with high resolution EM has provided several  
9 important advances in our understanding of the structure-related  
10 mechanisms by which the filtration occurs. Since direct, nano-scale  
11 observations of RO membranes in operating conditions are so far  
12 virtually impossible, we attempted to capture the changes in  
13 membrane behaviour using a tracer stain. The permeation through the  
14 PA layer depends on many factors, such as the accessible surface area  
15 of the PA layer and the nature of transport pathways within the PA  
16 layer and the 10 nm PA nano-film.

17 Using high resolution FIB-SEM 3-D reconstructions, we showed that  
18 the highly complex structures of the PA layer have a much larger  
19 active surface area than when examined via AFM. The behaviour of  
20 tracers within the dry and wet PA highlights the role of water  
21 solvation and swelling in activating the transport pathways. Finally, at  
22 the sub-nano-scale, we visualised <1 nm pore-like structures within

1 the PA nano-film using heavy metal staining. These observations lead  
2 to important conclusions about the membrane behaviour during the  
3 permeation and the role of water during this process.

#### 4 **4.1. 3-D reconstructions of the polyamide layer**

5 FIB-SEM was used to obtain 3-D reconstructions of membrane  
6 morphology with resolution comparable with the resolution of low  
7 magnification TEM imaging [14]. Discrete voids within the PA layer  
8 observed with FIB-SEM correspond in size (average 20 nm) to those  
9 observed in a previous electron microscopy studies [19,20]. We build  
10 on the aforementioned studies by focusing on reconstruction of the  
11 surrounding PA envelope and studying the distribution and  
12 interconnectivity of voids within the PA envelope. Additionally, the  
13 heavy metal staining methods used allowed for more robust and  
14 automated thresholding, which enabled a straightforward  
15 quantification of the structural parameters of the PA layer and void  
16 spaces.

17  
18 Large empty spaces under the ridges of the PA layer, which might be  
19 easily accessible to fluids (Figure 1D), as well as smaller, discrete  
20 voids within the PA envelope (Figure 2) were observed. While cross-  
21 sectional studies tend to assume all voids observed in the image are  
22 discrete [20], it is important to highlight that some of the voids within  
23 the PA layer, which appear discrete in the 2-D cross-sectional images,  
24 are open to either of the surfaces (but not both – Figure 1D, SI Movie  
25 1, Figure SI 4). The presence of large empty spaces under the ridges  
26 increases the active surface of the membrane, which in turn may  
27 affect the water flux. The range of morphologies of the protuberances  
28 and their impact on separation performance have been reported on in  
29 previous studies [9,37–40], but until now have not been accurately  
30 quantified. We calculated structural parameters of the PA layer such

1 as local thickness or relative area ratio and compared the results with  
2 AFM data.

3 It has been shown that while AFM is commonly used in the  
4 characterisation of RO membranes [37,41], it significantly  
5 underappreciates the complexity of the PA layer. Using a SW30HR  
6 membrane, we showed that the actual front surface area might be 2.5-  
7 4 times larger than when estimated with dry AFM. Similarly, the  
8 SA/V ratio might be 2-3 times greater. On the other hand, the  
9 morphology of the rear side agreed with previous studies [42] and the  
10 surface area of the rear sides of the PA layer was comparable between  
11 FIB-SEM and AFM measurements.

12 Structures observed with SEM could be linked with lower hierarchy  
13 structures observed with TEM to enable a multi-scale mapping and to  
14 establish a hierarchical model of RO membranes. Cross-sections of  
15 FIB-SEM reconstructions can be correlated to TEM micrographs  
16 (compare Figure 1C with Figure 4A and Figure 5A). It is important to  
17 note that FIB-SEM has limited capability to resolve the internal  
18 structures in the PA layer (i.e., stacked membrane folds or smaller  
19 voids), due to small contrast variations between the internal regions.  
20 Therefore, we propose FIB-SEM should be utilised as a tool to  
21 estimate the structural properties of the PA layer envelope (the  
22 volume within the external surface of the PA layer).

#### 23 **4.2. Nano-scale effects of staining**

24 RuO<sub>4</sub> stains polymers that contain aromatic rings, amine/amide  
25 groups or unsaturated carbohydrates [43]. While the bulk effects of  
26 RuO<sub>4</sub> staining are well known and described [43], experiments using  
27 RuO<sub>4</sub> to study permeation pathways into RO membranes, have not  
28 previously been conducted. By analogy to polyethylene [44,45], we  
29 suggest that RuO<sub>4</sub> would oxidise the carbonyl and amide groups of  
30 polyamide. While this process alters the chemical composition of the  
31 stained film, it fixes and stabilises the structure by forming additional

1 crosslinks between oxidised functional groups.  $\text{RuO}_4$  will move  
2 through the polymer network until it oxidises one of the chemical  
3 groups, limiting the pathway for diffusion of other molecules. At this  
4 stage, precipitates (larger clusters of ruthenium oxides) will form  
5 within the PA film. Therefore, the stain propagation is expected to be  
6 a function of diffusion and reactivity within the PA film. Upon  
7 membrane hydration, the staining of the film became more extensive  
8 (Figure 4D, Figure 7B, Figure 5B). In the aqueous environment, the  
9 PA film swells and enables further stain propagation within the  
10 membrane, facilitated by  $\text{RuO}_4$ -water interactions.

11

12 Nanocrystals of  $\text{RuO}_2$  were also found to precipitate on external  
13 surfaces of the polymer [46]. This phenomenon occurs for all  
14 staining conditions (wet and dry) and is expected upon reduction of  
15 unstabilised  $\text{RuO}_4$ . This process occurs alongside the film staining  
16 and increases with exposure time (SI Figure 7). A short exposure of 1  
17 min was selected to provide suitable contrast to image the  
18 nanostructure of the PA film.

19

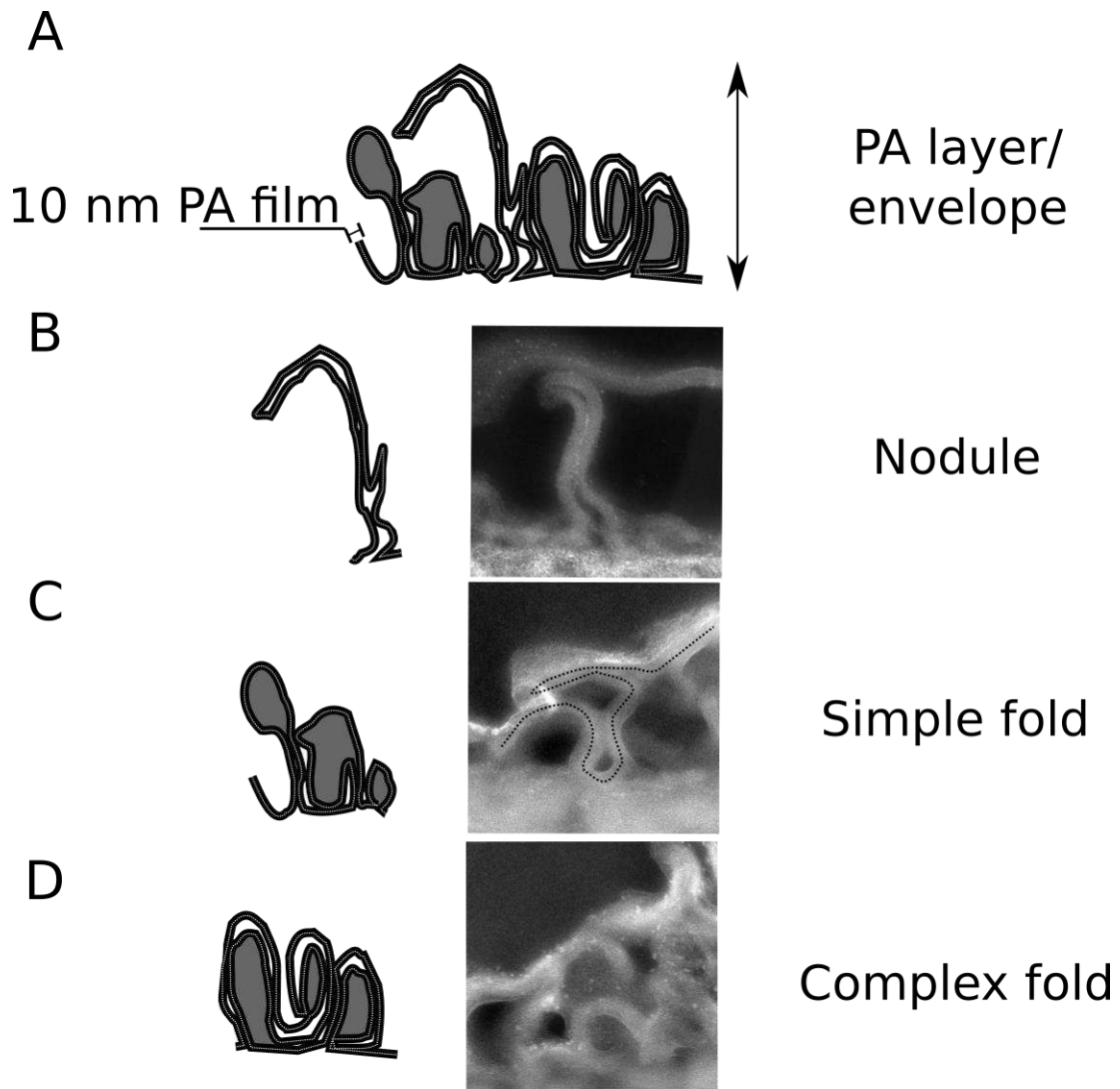
20 At higher magnification, TEM images of the PA layer cross-sections  
21 revealed the presence of “channels” within the PA structure. These  
22 structures were identified as hollow regions, possibly formed by tight  
23 folding of the PA film. The presence of “channels” was further  
24 confirmed by wet staining experiments. Staining patterns (Figure 5)  
25 suggest that many of the finger-like structures observed in the TEM  
26 images should be considered as “deflated balloons” rather than solid  
27 nodules. Staining of each individual “channel” may vary depending  
28 on its openness. Empty “channels” would be visible if the surrounding  
29 membranes hindered all diffusing  $\text{RuO}_4$ . Filled “channels” would  
30 form if some of the  $\text{RuO}_4$  passed through the PA film without  
31 oxidation of any functional group in the PA network and precipitated  
32 in the “channel” space. Importantly, “channels” were observed not  
33 only in the protuberances on the front side of the PA layer, but also in

1 the loops of the basal regions (Figure 5D). Regions, in which  
2 “channels” are visible, might have been formed from a highly  
3 crumpled single nano-film as suggested by Karan et al. [15]. In Figure  
4 9, a schematic of features observed in the PA layer is shown.

5  
6 A number of studies shows cave-like protuberances, which agrees  
7 with the crumpled nano-film model [6,16,19]. However, not all of the  
8 structures observed in the PA layer could be simply explained by film  
9 crumpling. Some of the studies report the presence of discrete voids  
10 [19,20]; in particular, Pacheco et al. [19] visualised these voids from  
11 reconstructed electron tomograms and attributed formation of  
12 discrete voids to the presence of aqueous droplets during interfacial  
13 polymerisation processes. Studies, in which this explanation of void  
14 formation was proposed previously [47,48], describe voids of  
15 micrometre (or greater) in size - orders of magnitude higher than our  
16 case.

17 The smallest voids reconstructed in our study were around 15 nm in  
18 diameter. Pacheco et al. also omitted reconstructing smaller voids due  
19 to difficulties with interpreting the contrast in the images and the  
20 resolution limit of electron tomography [49,50]. Smaller voids were  
21 definitely present, but it is not clear whether they were discrete or  
22 interconnected. We have made the same observation of both discrete  
23 and interconnected voids in our study. Future work using direct  
24 electron detectors and phase plates in electron microscopes will  
25 enable sufficient image resolution to establish whether some of these  
26 voids are in fact interconnected and to generate more complete model  
27 of the PA membrane.

28



1

2 **Figure 9** A schematic of features observed in 2-D cross-sections of  
 3 **the PA layer. (A) A general schematic of the ridge-valley**  
 4 **structure. Individual features: (B) a nodule, (C) simple fold and**  
 5 **(D) a complex fold. STEM images show fragments of the PA layer**  
 6 **from top to PSf interface (white dashed line). The black, solid line**  
 7 **represents the 10 nm crumpled, but continuous (dotted line)**  
 8 **nano-film. Regions in grey can be interpreted as discrete holes.**  
 9 **White regions within the PA layer are interpreted as “channels”.**

10 **4.3. Sub-nanometer porosity**

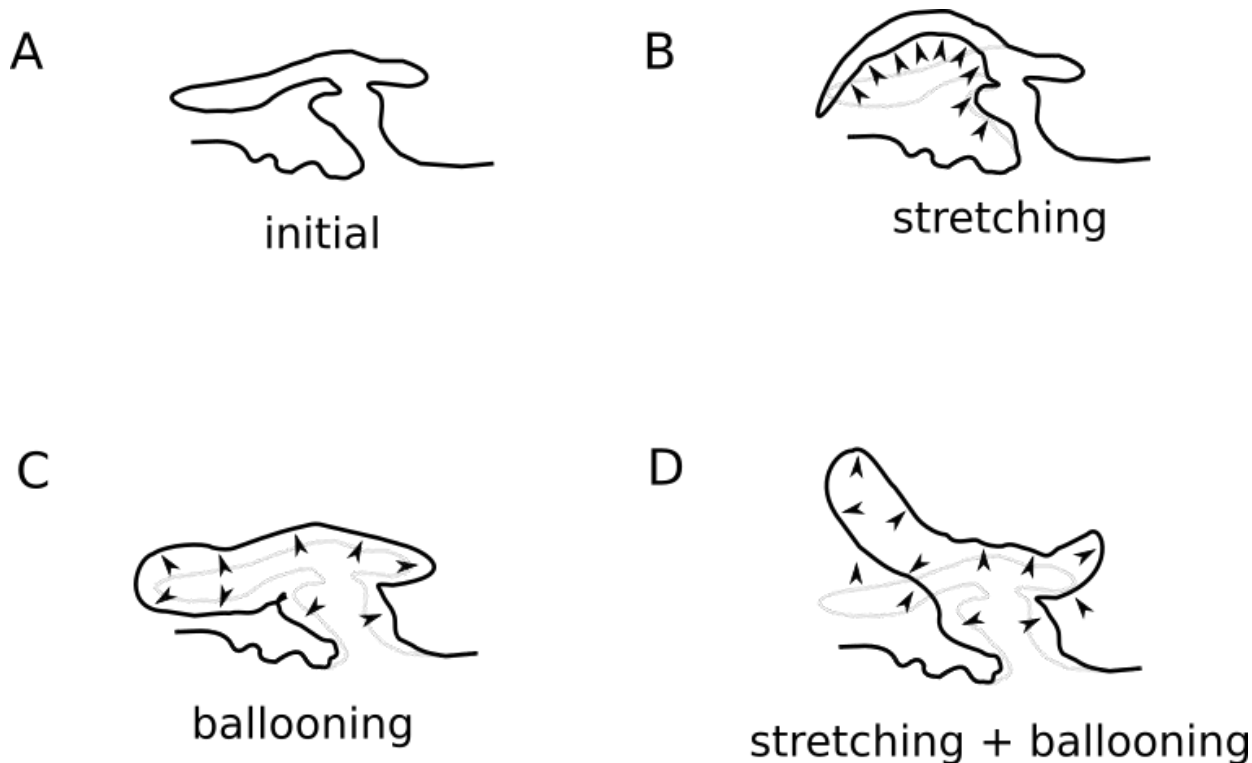
11 It has been suggested that nano-filtration through the membrane  
 12 occurs within the PA nano-film itself as a result of sub-nanometre  
 13 pores in the nano-film [4]. We attempted to visualise <1 nm diameter  
 14 permeation pores within the PA nano-film by imaging the precipitates

1 using high resolution HAADF-STEM. The diameter of observed stain  
2 precipitates within the PA nano-film matches the pore sizes estimated  
3 with PALS [22,25,28] and calculated from molecular dynamics [26].

#### 4 **4.4. Connections between structure and performance**

5 With the new data presented here we formulate a more accurate  
6 hypothesis about the behaviour of the PA structures in the aqueous  
7 environment. It was previously suggested that an increase in swelling  
8 correlates with increased permeability in membranes [37]. The  
9 reported swelling of the RO membranes (given as the difference  
10 between the thickness of wet and dry membranes observed in AFM)  
11 is usually in the range of 10-20 nm, possibly due to the rigidity of the  
12 cross-linked, aromatic PA [20,37,41]. If we take into account the  
13 morphology revealed by FIB-SEM, a few different mechanisms  
14 should be considered. These mechanisms may involve external-  
15 (stretching) and/or internally-driven (ballooning) expansion of the PA  
16 structures. Tentative mechanisms of swelling are sketched in Figure  
17 10.

18 In the present study, we were able to visualise the penetration of water  
19 in the PA by using a stain tracer and elucidated the impact of water on  
20 tracer transport within the PA. We concluded that not all of the PA  
21 surfaces are immediately accessible and confirmed that water plays an  
22 important role in activating the free volume of the PA layer and nano-  
23 film. In the case of stain permeation, two effects can be observed: the  
24 opening of <1 nm pores within the PA nano-film and the opening of  
25 the larger voids, which may be considered discrete in the dry state,  
26 within the PA layer. Both phenomena might, in turn, facilitate ion  
27 permeation through the PA layer.



1

2 **Figure 10 Possible responses of the PA structures to the presence**  
 3 **of water. (A) The initial dry state. (B) Stretching – water acts**  
 4 **externally, elongating some structures, but preserving the original**  
 5 **shape the PA layer. (C) Ballooning – water acts internal filling**  
 6 **and expanding the free volume of the PA layer. (D) Combined –**  
 7 **water acts externally and internally.**

## 8 **5. Conclusions**

9 In summary, the structural features of PA layer morphology in a  
 10 representative RO membrane at the micro- and nano-scale have been  
 11 visualised in 2-D and 3-D. The structural parameters of leaf-like  
 12 sheets at the micro-scale were quantified. Their relation to the  
 13 performance of membrane is discussed. Well-defined “channels”  
 14 within the nodules, which have not previously been reported on, were  
 15 imaged at the nano-scale. At the sub-nanometre scale, <1 nm pores  
 16 were imaged. We believe our nano-scale results support the  
 17 hypothesis that there is a nano-porous network underlying the  
 18 filtration mechanics of the polyamide layer, which may be visualised  
 19 using heavy metal staining. The staining in dry and wet conditions



1 also indicates the mechanism, by which water facilitates stain  
2 permeation through the PA layer. Tomographic studies of stained  
3 membranes provide new insight into the relationship between the  
4 structure and the membrane performance and open new possibilities  
5 for studying water flow and fouling in the vicinity of the PA layer in  
6 silico.

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12 Physical Sciences Research Council (EPSRC). We thank Prof.  
13 Enrique Gomez for his helpful discussions.

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