Size-selective separation of biologically relevant macromolecules using nanoporous channel membranes has for several years been a tempting topic in nanoengineering, chemistry, and medicine, as it allows for simple and direct purification of biomolecules such as proteins, as well as the mimicking of biological membranes.1–5

The efficiency of size-dependent separation is determined by the relation of pore size to the effective hydrodynamic diameter (or the Debye radius, respectively) of the targeted molecules in a given electrolyte. Small protein molecules were reported to pass through porous channels with approximately three times their diameter,4 though some unknown factors such as the physical shape of the protein, its 3-D hydrodynamic dimensions, and hydrogen bonding with water may affect the cutoff pore size.

To achieve the relevant pore sizes for protein separation in the range of a few nanometers, polymer type membranes (dialysis),6 high temperature silica pore segregation,5 or several approaches based on self-organized anodic porous alumina (with subsequent diameter reduction using metal7 or silica filling8) have been used.

In principle, a most elegant and effective way for membrane fabrication would be to use directly self-organized porous or tubular oxide structures that can be formed by anodizing suitable metals under optimized conditions.9–11 However, typical self-organized channels in anodic alumina or titania nanotube membranes are too large (∼20–200 nm in diameter) to be used for most cases of macromolecular separation.

In the present work, we demonstrate that a recently reported new anodization approach12,13 can be modified to produce titania layers with defined channels that are suitable for size selective protein separation. Free standing, flow-through membrane layers can be produced simply by complete anodization of thin Ti metal foils. A key advantage of these layers is their photocatalytic activity which can be used for effective declogging of the membranes. Clogging of membranes is a notorious problem occurring in virtually all size separation devices if the pore openings are within the size range of the smallest excluded proteins.

Figure 1a–c show a TiO₂ nanochannel membrane, prepared by complete anodization of a 10 µm thick Ti-foil in dehydrated glycerol-K₂HPO₄ solution at 180 °C (see experimental details in Supporting Information (SI)). The final point of the anodization process can easily be determined by recording the current during the process.

Figure 1a shows the foil after the anodization process. It is converted to an oxide layer of approximately 10 µm thickness (i.e., the oxidation efficiency for metal to oxide is in the range of ∼50%). The entire thickness consists of aligned interlinked regular channels with a width in the range of 8–12 nm (Figure S2). The TEM image in Figure 1b confirms the SEM and shows channel openings of ∼8–15 nm in diameter. A statistical evaluation of the size distribution of nanochannels from SEM and TEM analyses in Figure 1d gives an average pore size of 10 ± 2.4 nm for SEM and 11 ± 2.7 nm for TEM with a sharp cutoff of >14–15 nm.
diameters. That is, the anodization process produces a very defined and narrow distribution in pore sizes that is desired for a size separation membrane. The fact that the distribution obtained from TEM shows a slight shift toward higher openings can be ascribed to the TEM sample preparation by ion milling and stereological effects. While the HRTEM in Figure 1b shows mostly amorphous material to be present, the XRD pattern, taken over the entire membrane (Figure 1e), shows that the structure contains also anatase crystallites. Figure 1d demonstrates that comparably large-area membranes (1 × 1 cm²) can easily be fabricated and are highly water permeable.

To characterize the permeability of an as-formed sample for macromolecules, three biologically relevant proteins with different Stokes radii were selected [Cytochrome C (12 kDa), 1.63 nm; Bovine Serum Albumin (69 kDa), 3.62 nm; β-galactosidase (116 kDa), 6.86 nm]. Based on the channel size distribution (Figure 1d) one may expect an exclusion threshold in the range of a molecular diameter of 3–6 nm. For permeation measurements, membranes were clamped between O-rings of a two-chamber cell (a schematic diagram is given in the SI, Figure S3). The reservoir chamber is filled with a protein solution in phosphate buffered saline (PBS) and the outlet chamber with pure PBS. The content of protein that permeated through the membrane was quantified with a Micro BCA Protein Assay Kit (Pierce) after collecting samples at different time intervals (Figure 2) (see also the SI for details).

![Figure 2](image-url)  
**Figure 2.** Protein permeation through a 10 µm thick TiO₂ nanochannel membrane (as in Figure 1) vs time for three different size protein molecules: Cyt. C, BSA, and β-Gal. The dotted lines represent the initial slope of the curves.

From Figure 2 it is apparent that the membrane allows Cytochrome C and BSA to pass while, for the largest molecule β-galactosidase, no permeation could be detected even after 3 days of experiments. The permeation flux is clearly faster for small molecules, three biologically relevant proteins with different Stokes radii were selected [Cytochrome C (12 kDa), 1.63 nm; Bovine Serum Albumin (69 kDa), 3.62 nm; β-galactosidase (116 kDa), 6.86 nm]. Based on the channel size distribution (Figure 1d) one may expect an exclusion threshold in the range of a molecular diameter of 3–6 nm. For permeation measurements, membranes were clamped between O-rings of a two-chamber cell (a schematic diagram is given in the SI, Figure S3). The reservoir chamber is filled with a protein solution in phosphate buffered saline (PBS) and the outlet chamber with pure PBS. The content of protein that permeated through the membrane was quantified with a Micro BCA Protein Assay Kit (Pierce) after collecting samples at different time intervals (Figure 2) (see also the SI for details).

![Figure 3](image-url)  
**Figure 3.** Clogging of membrane by extended permeation and reopening by photocatalytic effect. (a) BSA exposure of the TiO₂ nanochannel membrane resulting in reduced flux and after certain time (120 h) to complete clogging. After 160 h the membrane was UV exposed leading to reopening; inset: BSA transport rate. (b) XPS analysis for C1s and N1s peaks of clogged and declogged membrane (after UV exposure).

In conclusion, the present work shows a cheap and effective method to produce free-standing, self-cleaning membranes for size selective macromolecular separation. The nanochannel TiO₂ membrane structure with both sides open can be formed in a single anodization step of a Ti-foil and contains regular channels 8–12 nm in diameter. It should be noted that the size and distribution of the channels formed in our anodic layers are among the smallest and narrowest reported in literature for any self-organized anodic system. In light of considerable work on TiO₂ nanotube membranes and the photocatalytic ability of ordered titania structures, it should be mentioned that the material hereafter formation already contains anatase crystallites that provide considerable photocatalytic activity; i.e. the material does not need to be annealed which avoids the danger of heat induced crack formation in the membrane production process. Further optimization of the throughput and the declogging efficiency may be achieved by reducing the layer thicknesses combined with patterning of the structures.
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Supporting Information Available: Experimental details, XPS analysis, schematic diagram, optical images of the samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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