SUPPORTING INFORMATION

Flow-Based Assembly of Layer-by-Layer Capsules through Tangential Flow Filtration

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SUPPORTING METHODS

Materials. Poly(styrene sulfonate) (PSS, M_w ~70 kDa), poly(allylamine hydrochloride) (PAH, M_w ~58 kDa), poly(diallyldimethylammonium chloride) (PDADMAC, M_w ~200-350 kDa), fluorescein isothiocyanate (FITC), ethylenediaminetetraacetic acid (EDTA), hydrofluoric acid (HF), NaCl, HCl, NaOH, $CaCl_2$, Na_2CO_3 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Doxorubicin HCl (DOX) was purchased from OChem Incorporation (Chicago, IL, USA). Ultrapure water with a resistivity greater than 18 MΩ·cm, from an inline Millipore RiOs/Origin purification system (Millipore; Billerica, MA, USA) was used. Silica particles with diameters 2.39 µm, 1.04 µm, 889 nm, 519 nm, and 177 nm were obtained from Microparticles GmbH (Berlin, Germany). The components for the flow-based tangential flow filtration (TFF) system were purchased from Spectrum Labs (KrosFlo Research IIi Tangential Flow Filtration System; Spectrum Labs; Rancho Dominguez, CA, USA) which included tubing (size 14, Masterflex, Pharmapure). TFF filter modules made of modified (proprietary) polyethersulfone with a pore size of 300 kDa, 750 kDa or 0.2 µm were obtained from Spectrum Labs (Microkros, 20 cm^2 surface area). The product code for one of the typical TFF filter modules used is C02-E20U-05-N. Full technical specifications (module type, effective length, membrane type, MWCO rating, full dimensions, packaging conditions, technical drawings and schemes etc.) are available online from the manufacturer (Spectrum Labs, http://www.spectrumlabs.com/). PAH, PSS and PDADMAC solutions were prepared at 1 mg mL^{-1} with 0.5 M NaCl and pH was adjusted to ~ 6.0–6.5 using HCl or NaOH. FITC was added into a 1 mg mL^{-1} solution of PAH and brought to high pH (~ 9-10) for an hour to allow for a roughly 1:500 functionalization with FITC. The pH of PAH-FITC was then returned to ~ 6.0–6.5 before use. PSS-stabilized porous $CaCO_3$ particles with a diameter of ~5 µm were produced as previously described.[1] The same batches of particles and polymer solutions were used for all comparative experiments.

Table S1. TFF filter modules and particles. The TFF filter modules used in this study had three different pore sizes, two of them supplied by the manufacturer with molecular weight cut-off (MWCO) values and one supplied with a pore size value in micrometers. The manufacturer provides a "pore size chart" (available on the manufacturer's webpage: http://www.spectrumlabs.com/) that can be used to approximate dimensions of pore sizes for filters with MWCO values. "Suitable particle diameter" is determined using the guideline that particles need to have a diameter of at least around five times the diameter of the pores to be efficiently retained.

TFF filter module	**Typical diameter of pores according to "pore size chart"**	**Suitable particle diameter**	**Template sizes used in this study**
300 kDa	~ 20 nm	≥ 100 nm	177 nm
750 kDa	~ 60 nm	≥ 300 nm	889 nm, 519 nm
0.2 µm	~ 200 nm	≥ 1 µm	2.39 µm, 1.04 µm

Core removal. Calcium carbonate cores were dissolved with EDTA (200 mM, pH 8.0) or sodium acetate buffer (40 mM, pH 4). Silica cores were dissolved with 5 M hydrofluoric acid (HF). *Caution: HF is dangerous and should be handled with caution.* Capsules were then washed three times with ultrapure water.

Centrifugation-based LbL. Centrifugation-based LbL assembly was performed using standard methods.[2-4] Specifically, the procedure was:

1. 300 µL of 50 mg mL^{-1} silica particles (889 nm) was placed in a 1.7 mL microcentrifuge tube and mixed with 700 µL of ultrapure water.
2. Sample was washed three times with ultrapure water:
    a. Centrifuge: 1200 rcf, 30 s
    b. Supernatant was carefully aspirated (to not disturb the pellet) until ~ 50-100 µL solution remained.

c. The particles were resuspended in the remaining solution and then mixed with 900 µL fresh ultrapure water.

3. After the last washing step, 1 mL of PAH solution was added to the particles.
4. Polymer was allowed to adhere to surface of the particles for 15 min with constant agitation through rotation.
5. Particles were washed three times with ultrapure water (as step 2 above).
6. Step 3-5 were repeated with PSS solution, then again with PAH solution etc., until four bilayers had been deposited. One deposition cycle of PAH and PSS constitutes a bilayer.
7. The finished particles were washed three times with ultrapure water (as step 2 above).

Flow-Based TFF LbL. See Figure S2 for the schematic to be used with these instructions, Figure 5 for a simplified overview and Figure S3 for additional details for a fully automated system.

1. Make sure the loop has been washed/rinsed thoroughly.
2. Load the surge tank with particle suspension. Typical amount of particles used: 300 µL, 50 mg mL^{-1}. Pre-wash and/or sonicate particles before starting if necessary.
3. Add polymer A to suspension. Typical value: 4 mL of polymer A (e.g., PAH).
4. Install the surge tank.
5. Adjust valve-1, 2 to put layering loop in service. Washing loop should be completely isolated at this step.
6. Adjust the set point of pump flow rate. Typical value: 50 mL/min.
7. Start the pump.
8. Monitor the feed and retentate pressure for some seconds to make sure the layering loop is properly in service.
9. Record the time. Let the system run for a desired period of time. Typical value: 15 min layering.
10. Disconnect the pump suction line and let the loop discharges in surge tank via return line. As a measure of an empty system, feed line pressure should reach its minimum value. Typical value: 0.1 psig.

11. Stop the pump.
12. Connect the suction line and normalize the loop.
13. Re-adjust the valve-1, 2 to put washing loop in service.
14. Check both valve-3 and permeate valve to be open.
15. Start the pump.
16. Inject wash solution in surge tank for dilution. Typical value: 10mL of ultrapure water.
17. Check the permeate stream to see if polymer separation rate is reasonable.
18. Monitor the level of liquid in surge tank. Wait until the level reaches to a desired volume. Typical value: 10-30 s.
19. Stop the pump.
20. Close valve-3.
21. Inject wash liquid via permeate stream. Typical value: 12.5 mL of ultrapure water. This volume should be collected in surge tank.
22. Open valve-3.
23. Start the pump. Repeat the process from step 17 if washing is not adequate. Typical value: two more times.
24. Stop the pump. Close valve-3.
25. Depending on the volume of particle suspension to be recovered, use a small syringe and connect it to filter backwash/permeate stream (typical: 3 mL) and proceed with backwash and particle collection
26. If required: take sample for zeta potential measurement, flow cytometry measurement etc.
27. Go to step 3 and repeat the process for polymer B (e.g., PSS). If 2× concentration of polymer is used add equal amount to particle suspension to achieve 1× concentration.

Quantification of PAH-FITC in permeate. A calibration curve for PAH-FITC was prepared through serial dilutions. The permeate from the TFF LbL system was collected continuously during

the washing step into 96-well plates. The polymer concentration was then analyzed using a multimode microplate reader (Infinite 200 PRO NanoQuant; Tecan, Männedorf, Switzerland).

SUPPORTING FIGURES

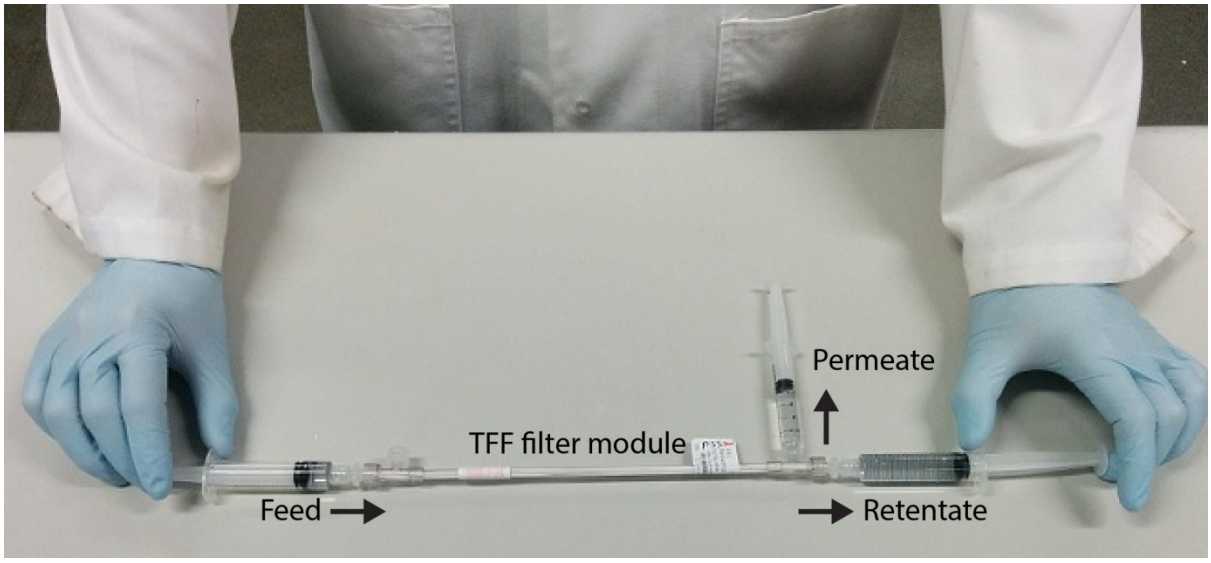

Figure S1. Manual washing using a TFF filter module. The particle suspension is pushed back and forth using the left and right hand syringes. Permeate is collected in the third (upper) syringe.

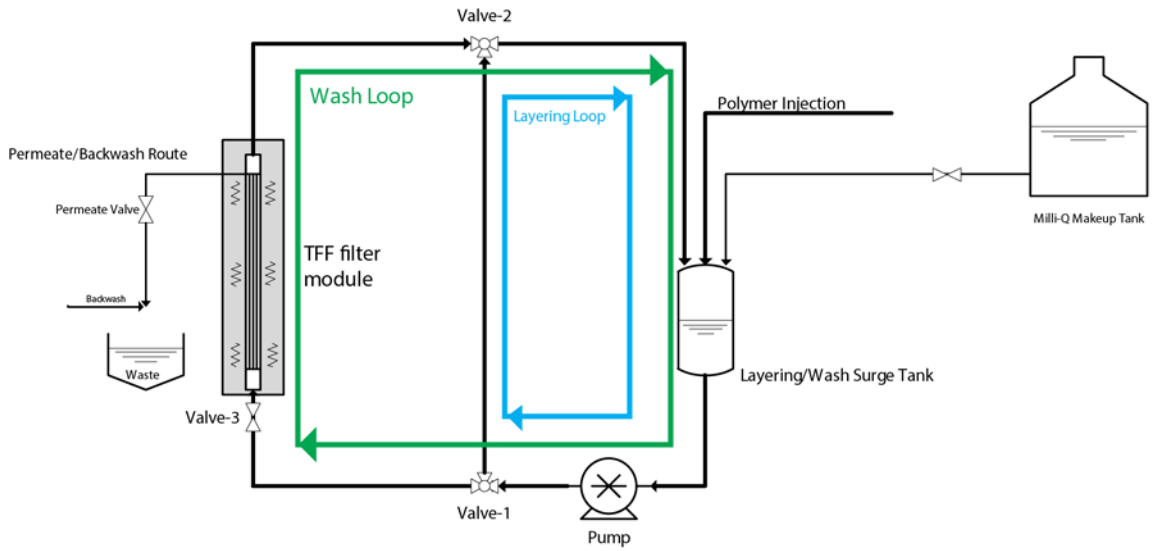

Figure S2. Lab-scale testing of flow-based TFF LbL system. See "*Flow-based TFF LbL*" (Supporting Methods) for details. See Figure S3 for fully automated TFF LbL system.

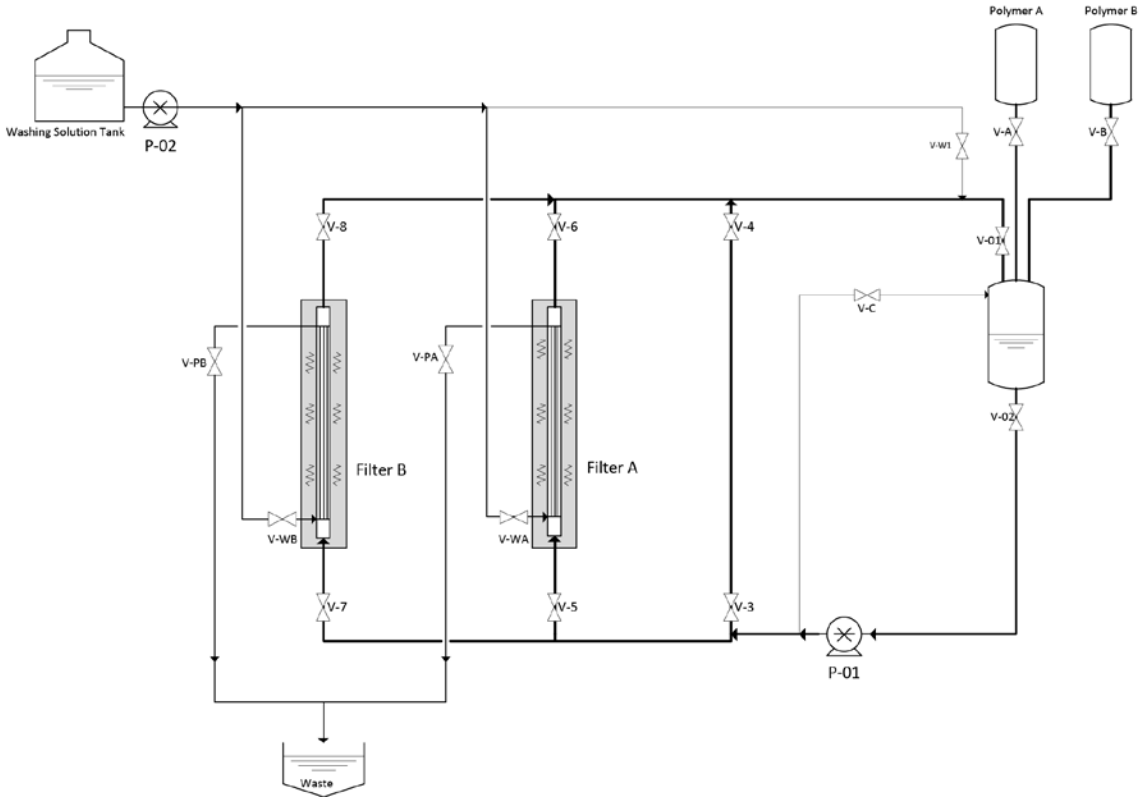

Figure S3. Design for fully automated TFF LbL system. Filter A is used for washing after layering of polymer A (e.g., PAH) and filter B is used for washing after layering with polymer B (e.g., PSS), the use of two filters minimizes the build-up of LbL films inside the filter. See Figure S2 for design for lab-scale testing.

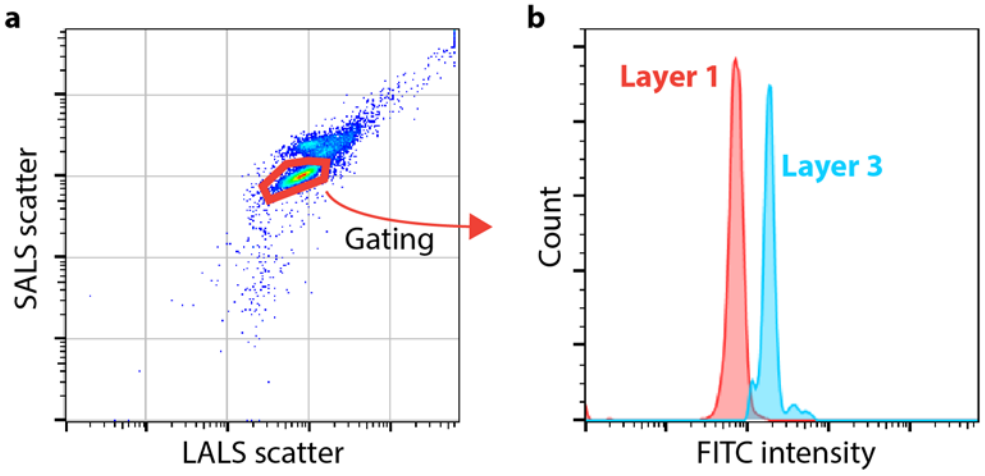

Figure S4. Analysis of fluorescence intensity of PAH-FITC/PSS layered particles using flow cytometry. **(a)** Particles are first identified and gated using scatter signals that exclude aggregates and debris. Around 90% of the total event count was inside the gate defining particles here. **(b)** The FITC signal from PAH-FITC (odd numbered layers) was then analyzed on the gated particles.

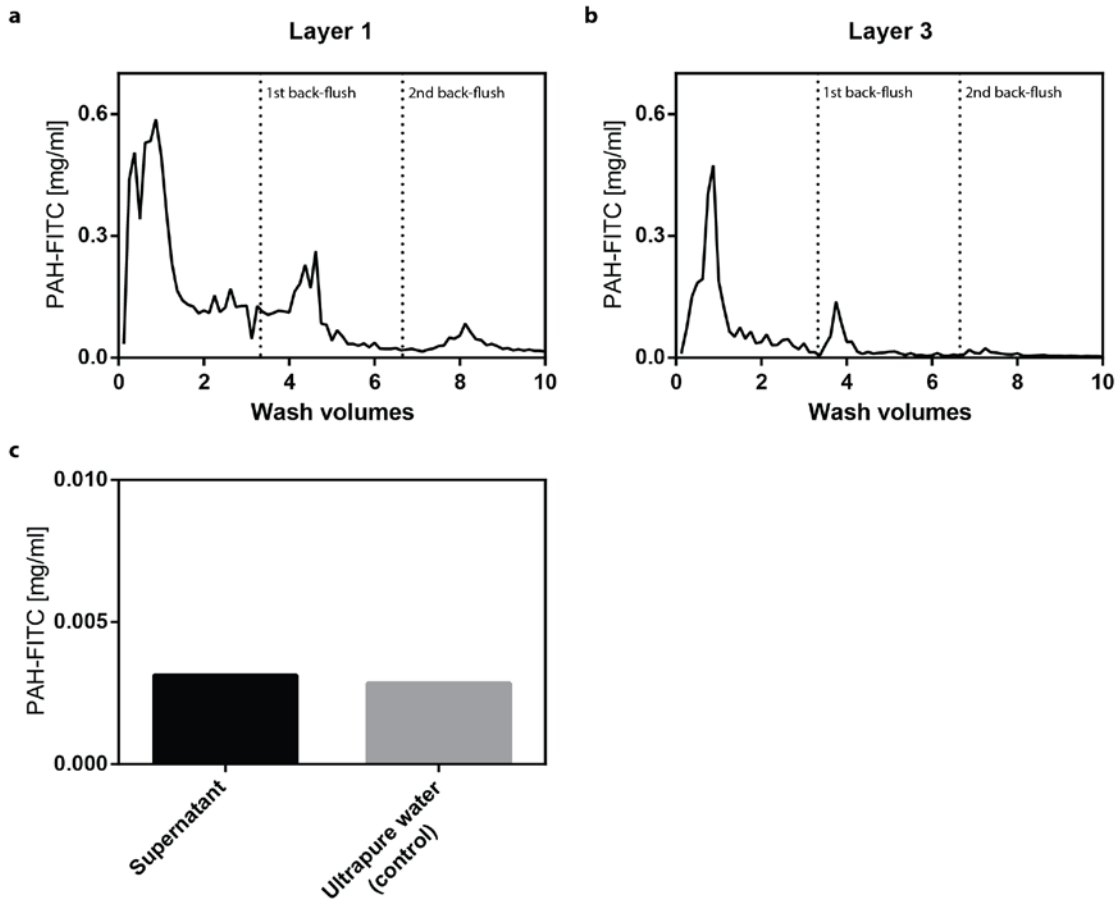

Figure S5. Removal of free polymer in solution during washing in the TFF LbL system. Silica templates 889 nm in diameter were layered with PAH-FITC and PSS, as outlined under "Flow-based TFF LbL" in Supporting Methods. **(a)** Quantification of PAH-FITC in permeate during washing after the first PAH layer (procedure described in Supporting Methods). Additional washing buffer is added first through dilution of the working volume and then through back-flush steps (as described in Supporting Methods). The amount of washing performed is expressed as "wash volumes", a common term in continuous-flow filtration and diafiltration, which is defined as volume of wash buffer added divided by the batch volume. **(b)** Quantification of PAH-FITC in permeate during washing after the second PAH layer (PAH/PSS/PAH, i.e., layer 3). **(c)** Quantification of PAH-FITC remaining in supernatant after TFF LbL washing of PAH/PSS/PAH-coated particles. Note that the detected amount is virtually at the background/noise level (compared to control sample of ultrapure water). Additional information regarding washing efficiency, recommended wash volumes etc. when performing TFF can be found in the manual and the application notes for the "KrosFlo Research IIi TFF System" available from the manufacturer's webpage: http://www.spectrumlabs.com.

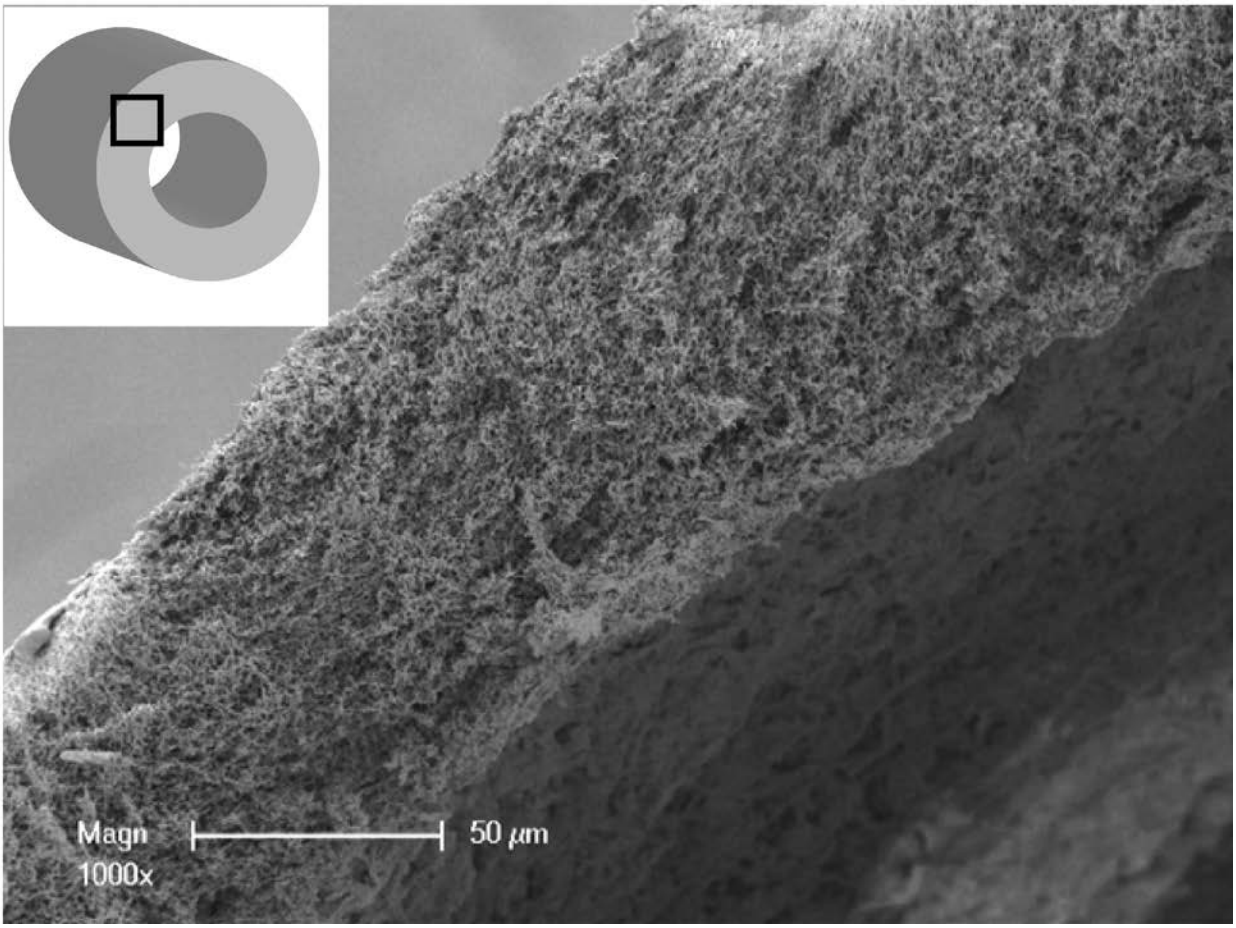

Figure S6. Scanning electron microscopy (SEM) image of cross-section of side-wall of a TFF filter module fiber with pore-size 0.2 µm. The inset is a schematic of approximately where (black box) on the fiber the SEM was obtained. Full technical specifications (including technical drawings/schematics) of filter modules are available from the manufacturer (Spectrum Labs, http://www.spectrumlabs.com/). Scale bar is 50 µm.

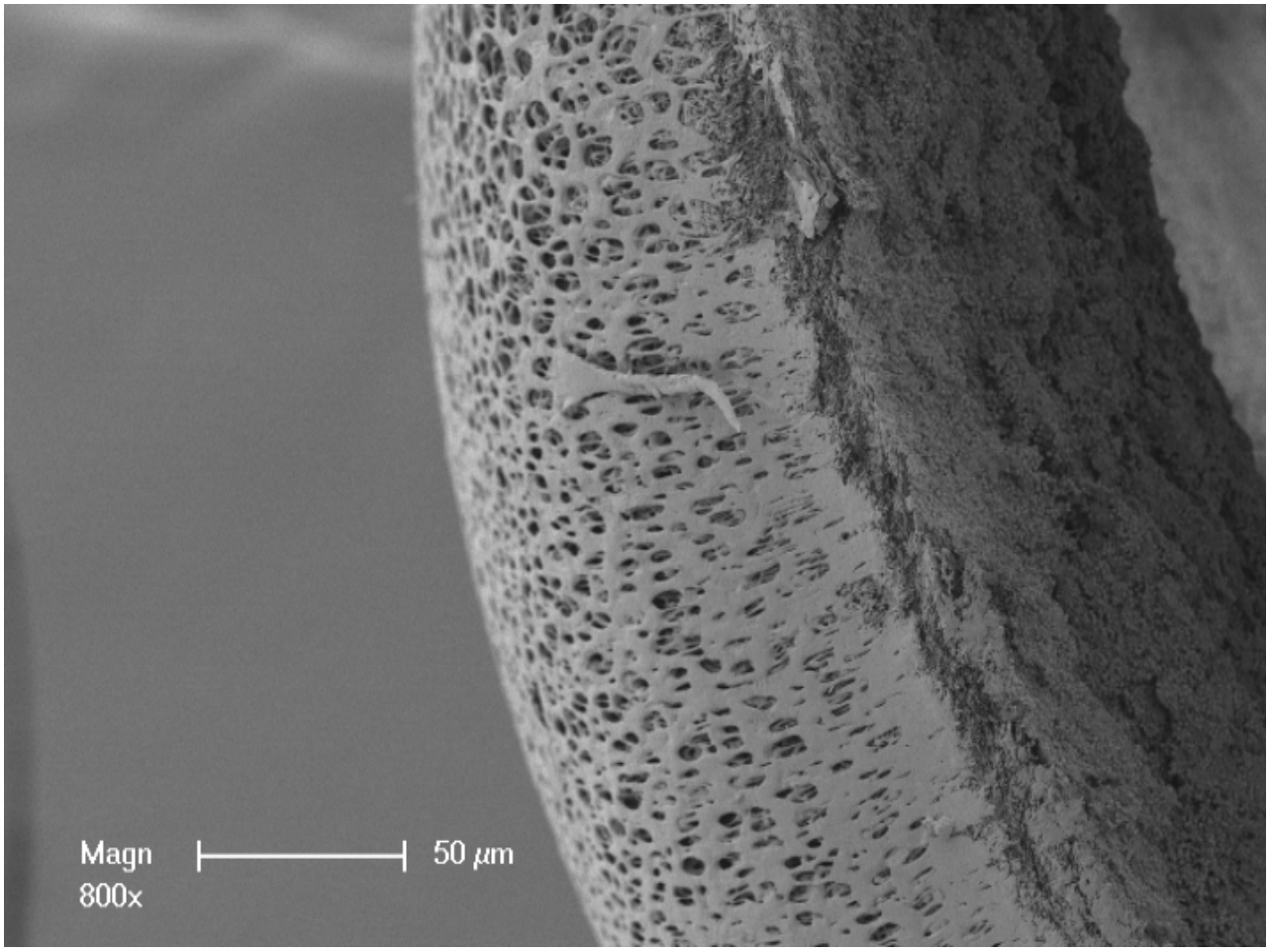

Figure S7. SEM image of cross-section and "outside" (permeate side) of a TFF filter module with pore-size 0.2 µm. Full technical specifications (including technical drawings/schematics) of filter modules are available from the manufacturer (Spectrum Labs, http://www.spectrumlabs.com/). Scale bar is 50 µm.

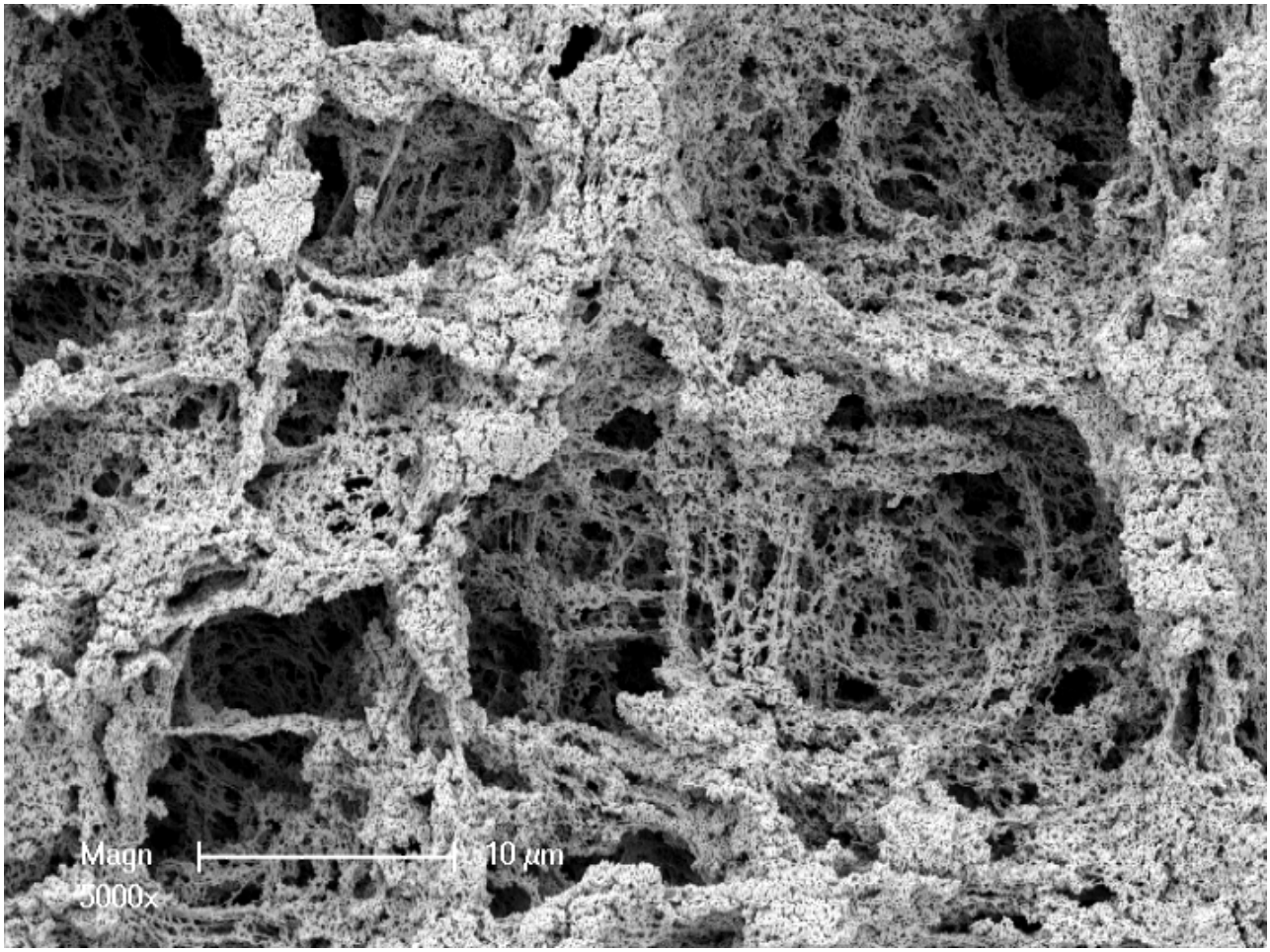

Figure S8. SEM image of unused TFF filter with pore-size 0.2 µm, close-up of lumen side. Full technical specifications (including technical drawings/schematics) of filter modules are available from the manufacturer (Spectrum Labs, http://www.spectrumlabs.com/). Scale bar is 10 µm.

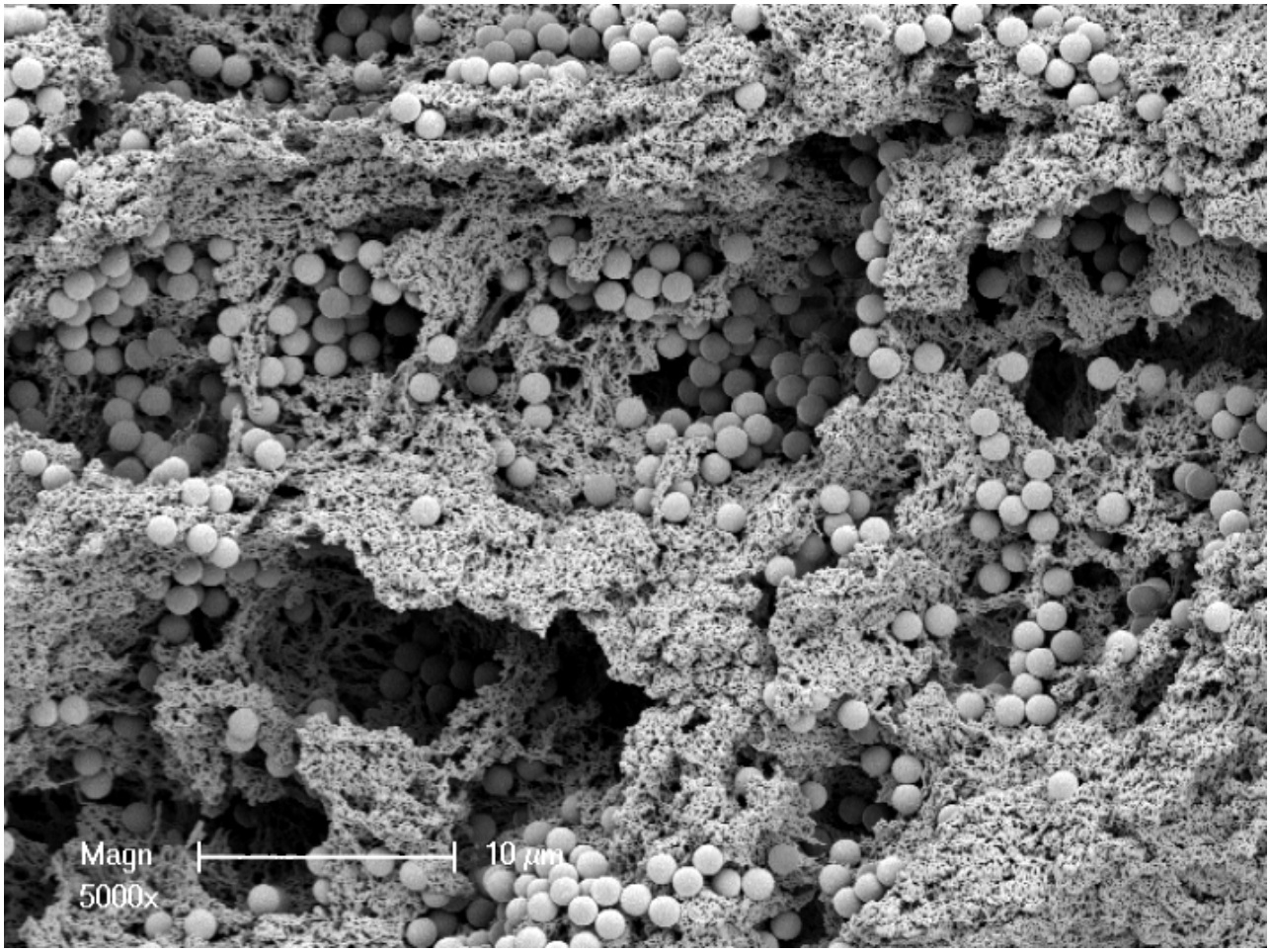

Figure S9. SEM image of used TFF filter with pore-size 0.2 µm, close-up of lumen side. Silica particles coating the surfaces in the filter are clearly visible. Full technical specifications (including technical drawings/schematics) of filter modules are available from the manufacturer (Spectrum Labs, http://www.spectrumlabs.com/). Scale bar is 10 µm.

Video S1. Demonstration of manual TFF washing using syringes and a TFF filter module. See Figure S1 for labels for each component. This video is available free of charge via the Internet together with the article at http://pubs.acs.org.

References

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