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High Throughput Synthesis and Screening of New Protein Resistant Surfaces for Membrane Filtration

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A novel high throughput method for synthesis and screening of customized protein-resistant surfaces was developed. This method is an inexpensive, fast, reproducible and scalable approach to synthesize and screen protein-resistance surfaces appropriate for a specific feed. The method is illustrated here by combining a high throughput platform (HTP) approach together with our patented photo-induced graft polymerization

Additional Supporting Information may be found in the online version of this article.

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(PGP) method developed for facile modification of commercial poly(aryl sulfone) membranes. This new HTP-PGP method was validated by comparison with our previous published results obtained using a bench-scale filtration assay of six well-studied monomers. Optimally-performing surfaces for resisting a model protein, bovine serum albumin (BSA), were identified from a library of 66 monomers. Surfaces were prepared via graft polymerization onto poly(ether sulfone) (PES) membranes and were evaluated using a protein adsorption assay followed by pressure-driven filtration. Bench-scale verification was conducted for selected monomers using HTP-PGP method; a good correlation with HTP-PGP results was found. © 2009 American Institute of Chemical Engineers *AIChE J,* 56: 1932–1945, 2010

Keywords: high throughput, protein-resistant surfaces, photo-induced graft polymerization, bovine serum albumin, membrane filtration

Introduction

Customizing surfaces for particular applications has been pursued for a long time with respect to selecting protective coatings for objects placed in aqueous (i.e., marine and medical) and air environments. The goals are usually to minimize the interaction of unwanted dispersed dissolved solutes and suspended particles with particular surfaces. Here, we are concerned primarily with proteins and protein aggregates. The search for customized protein-resistant or -repellant surfaces has been limited due to (i) the lack of widely accepted rules guiding the choice of such surfaces, (ii) the extremely diverse characteristics of proteins (i.e., multiple interactive forces acting simultaneously), and (iii) the enormous expense and time needed to find and develop new materials with optimal interfacial characteristics. Some success has been made as exemplified by the discovery and application of polyethylene glycol-coated surfaces to minimize protein adhesion and the trend to use hydrophilic (e.g., regenerated cellulose, polyamide) in place of hydrophobic [e.g., poly(aryl sulfone), poly(vinylidene fluoride), poly(propylene)] polymeric surfaces as synthetic membranes during pressure-driven membrane filtration. However, many of these surfaces suffer from other deficiencies, including negative influence on protein secondary structure, susceptibility to large pH changes, and in the case of membranes, low permeation fluxes.

Hence, developing new polymeric materials with appropriate surface or functional characteristics for different membrane filtration applications involves great effort and expense and often takes many years. More importantly, surface science has not yet developed to the point that allows prediction of the surface or functional characteristics needed to minimize undesirable interactions with solution components, and thus to control fouling. As a result, over the past 30 years, few new polymers have been used for membrane production. What is urgently needed is a fast, efficient, and reproducible process to allow quick selection of the best polymeric surface, and subsequent analysis of its mechanism of action, to gain understanding for future design of surfaces for membrane and other applications.

Here, we offer such a method, by adapting high throughput platform (HTP) approaches successfully used in chemistry (e.g., combinatorial spot/well analysis)[1] and biology (e.g., phage display[2] and SELEX[3]) to the facile modification of PES, using a HTP together with our patented photo-induced graft polymerization (PGP) method.[4–12] We illustrate the method by modifying commercial PES membranes, which affords a rapid assessment of protein/surface interaction in terms of filtration resistance developed after protein adsorption. In the PGP method, depicted schematically in Figure 1, poly(aryl sulfone) membranes are UV-irradiated, cleaving trunk polymer chains and forming reactive radical sites. Either water or methanol-soluble vinyl monomers covalently bond to these radical sites and undergo free-radical polymerization.[4] We call the combined method HTP-PGP.

Although HTP approaches have been used to evaluate process variables in the development of filtration processes using commercial available filtration membranes,[13,14] and to optimize membrane casting dope composition,[15–17] we report here for the first time the use of a HTP approach to modify polymer surfaces. The novel method proposed here proffers an inexpensive, fast, simple, reproducible, and scalable procedure to synthesize and screen protein-resistance surfaces by modifying poly(aryl sulfone), which has excellent physical and transport characteristics but poor surface chemistry. The method is, however, limited as it does not incorporate surface mixing or tangential flow to effect mass transfer, has very small test areas that could effect averaging of results and direct assaying of the grafted polymer on the membrane surface (i.e difficult to measure contact angle, XPS or ATR/FTIR directly), is incapable of allowing cleaning by back-washing, and, until now, is restricted to grafting of vinyl monomers with PES. We are currently addressing several of these limitations.

Previous work in our laboratory and that of other groups in academia and industry has approached this problem in the reverse order, by first choosing a priori a few "attractive" monomers (based mostly on intuition and the assumption that hydrophilic monomers with hydroxyl or ethylene glycol groups are best), conducting confirmatory studies to verify grafting and then testing filtration efficacy. The process has been slow, with a low probability of success and with little mechanistic insight. Our new approach of high throughput "reverse engineering" substantially increased the chances of finding grafted monomers with superior filtration performance. This process, if designed carefully and with a sensitive relevant assay, should provide several so-called winners that can then be verified at the bench scale. The approach itself provides mechanistic insight by generating a large quantity of data on structural properties and performance. Such data allows one to search for rules on mechanism, explaining why one surface is better than another, that can guide the choice of protein-resistant membranes.

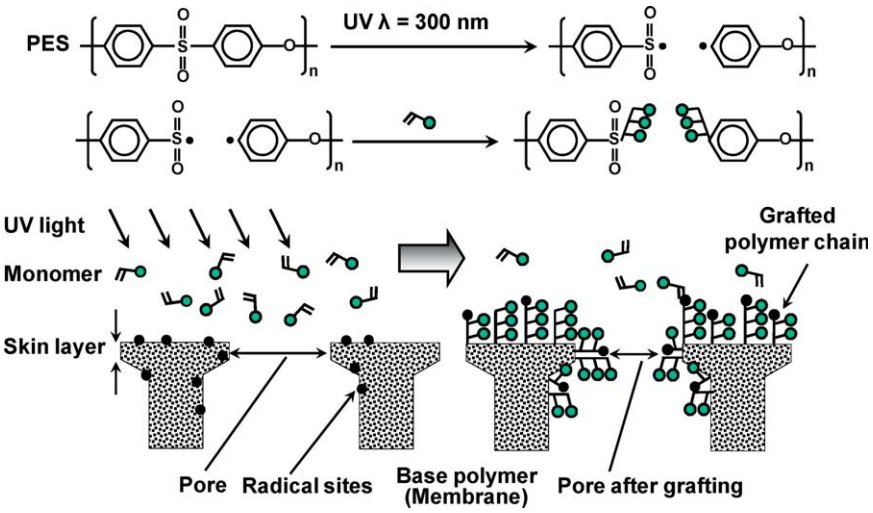

Figure 1. Graft Mechanism: In the PGP method, poly(aryl sulfone) membranes are UV-irradiated ($\lambda \approx 300$ nm), cleaving trunk polymer chains and forming reactive radical sites.

Either water or methanol-soluble vinyl monomers chemically bond to these radical sites and undergo free-radical polymerization. After Yamagishi et al.[4]

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Strategy

The strategy for membrane material and process development using reverse engineering HTP-PGP is depicted schematically in Figure 2, and a schematic illustration of HTP-PGP experimental setup is shown in Figure 3. First, an initial monomer library was chosen from a pool of likely candidates. The initial monomer library used in this work, shown in Figure 4, represents 66 monomers that are commercially available, categorized into nine groups based on chemical functionality. These monomers were then used to modify PES surfaces using the HTP-PGP approach. Candidate surfaces were synthesized, characterized in terms of permeability, and then screened with a protein solution challenge in the same multi-well filter plate.

We have successfully used two different screening assays to evaluate membrane performance: resistance to buffer filtration after protein adsorption by of graft-modified

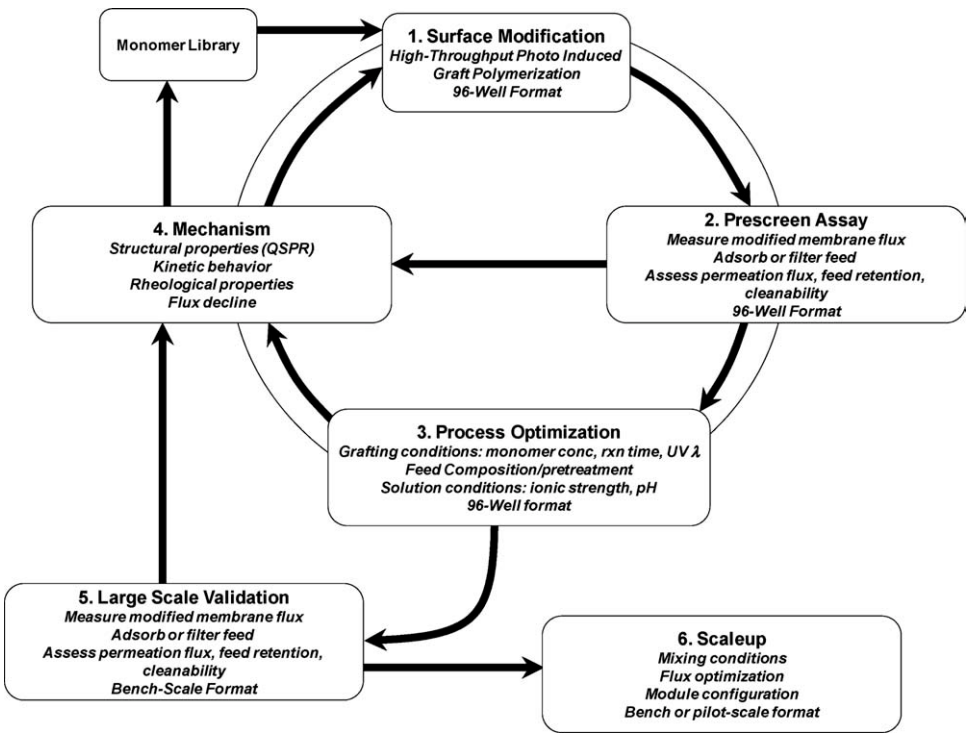

Figure 2. Surface material and process development using the "reverse engineering" HTP-PGP strategy with a membrane filtration as both an assay and as an example application.

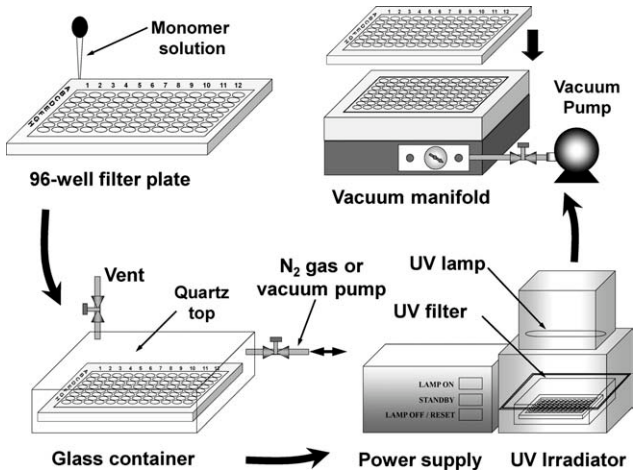

Figure 3. Schematic illustration of HTP-PGP experimental setup.

After Zhou et al.[18]

membranes, and resistance to protein solution filtration by of graft-modified membranes. The membrane-based approach, illustrated here, allows determination, in the HT platform, of performance parameters specific to membrane applications, including permeation flux, flux decline due to feed solute interactions with the membrane, feed component retention, and cleanability. Surface characterization such as contact angle and chemical composition is also possible.[19]

Here, we report on (i) validation of the HTP-PGP approach by comparison with previous results of PES grafted with six different monomers using a bench-scale method described by Taniguchi and Belfort,[8] (ii) screening of membrane surfaces, prepared from an initial monomer library of 66 commercial vinyl monomers, for their ability to resist interaction with BSA, (iii) verification of surfaces identified in the HT format via bench-scale filtration with mixing, and (iv) comparison of our results with those previously reported in the literature. Other feeds, including soil humic acid as model natural organic matter (NOM) hen egg white lysozyme, Chinese Hamster Ovary (CHO) supernatant, and immunoglobulin G (IgG) precipitate in the presence and absence of BSA, have also been evaluated using this method and the results are reported elsewhere.[20] We have also used the HTP-PGP approach to identify surfaces that resist fouling by water containing natural organic matter (e.g., humic and fulvic acids).[18] The HTP-PGP approach to synthesize and screen fouling-resistance surfaces is general, and thus provides the capability to develop surfaces optimized for specific feeds.

Experimental

Materials

Membrane. Polypropylene 96-well filter plates (Seahorse Labware, Chicopee, MA) were used in HTP-PGP experiments. A 100 kDa cut-off PES membrane coupon (effective area 19.35 mm^2) was mounted by the manufacturer on the bottom of each 400-μL well. The hydraulic resistance of the 96 membranes ranged from 8.12×10^{11} to 9.49×10^{11} m^{-1} with a coefficient of variation equal to 4.0%. In bench scale studies, a commercial PES ultrafiltration membrane (PBHK Biomax 100 kDa, Millipore Corporation, Bedford, MA) was used. Before use, the membrane sheets and filter plates were washed several times with deionized (DI) water and then soaked in DI water overnight to remove surfactant from the membrane coupons.

Monomers. Commercial vinyl monomers (66 total, Figure 4) were purchased from Sigma-Aldrich (Saint Louis, MO) and were used as-received without further purification. The name, structure, and formula weight (FW) of these monomers are shown in Supporting Information Table S1. These monomers were either dissolved in reagent grade water or ethanol (see Figure 4 and Supporting Information Table S1) depending on their solubility. Six of these

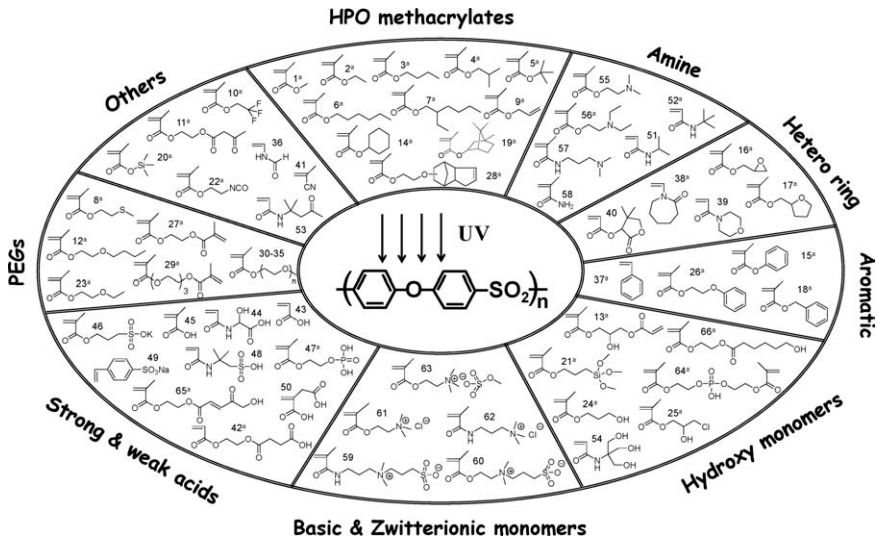

Figure 4. Initial monomer library: Library consisted of 66 monomers in nine groups. Numbers refer to Table S1 in the Supplemental Information.

Superscript "a" indentifies ethanol soluble monomers. After Zhou et al.[18]

monomers were used for HTP-PGP validation: *N*-vinyl pyrrolidinone (NVP), 2-hydroxyethyl methacrylate (HEMA); acrylic acid (AA), 2-acrylamidoglycolic acid (AAG), 3-Sulfopropyl methacrylate (SPMA) and 2-Acrylamido-2-methyl-1-propanesulfonic acid (AMPS). These monomers were dissolved in reagent grade water for grafting. An additional 62 commercial vinyl monomers were evaluated further. Unless otherwise noted, a monomer concentration of 0.2 mol L^{-1} was used for grafting experiments. Several monomers were evaluated at the bench-scale to verify HTP results, including selected promising monomers from HTP-PGP experiments that were representative of several different groups, a few monomers that did not perform well, and a few that performed well with other feed solutions. The same monomer concentration (0.2 mol L^{-1}) as that used at the HTP scale was used for bench scale verification. The monomers were dissolved in either water or ethanol depending on their solubility, as done in the HTP-PGP experiments.

Protein Feed Solution. Bovine serum albumin was chosen as a model protein to assess membrane fouling for both HTP and bench scale experiments. BSA (MW = 67 kDa, pI = 4.7) is negatively charged under our experimental conditions. Solution was prepared by dissolving BSA into phosphate buffered saline (PBS) solution to yield a protein concentration of 1 g L^{-1}. PBS buffer solution contained 10 mmol L^{-1} phosphate buffer, 2.7 mmol L^{-1} potassium chloride, and 137 mmol L^{-1} sodium chloride with pH 7.4 at 25°C. BSA and PBS tablets were purchased from Sigma-Aldrich (Saint Louis, MO).

HTP-PGP methods

Preparation of Modified Surfaces. The membranes on the 96-well filter plates were modified using the UV-induced graft polymerization method; mechanisms have been described in our previous publications.[4,6,21] The approach is shown schematically in Figure 3. UV irradiation was conducted in a chamber (F300S, Fushion UV Systems, Inc. Gaithersburg, MD) containing an electrodeless microwave lamp (~7% of the energy was at <280 nm). A bandpass UV filter (UG-11, Newport Corporation, Franklin, MA) was placed between the 96-well filter plate and the UV lamp to reduce the energy at wavelengths below 280 nm to <1%.

The membrane modification consisted of the following steps. After washing, the hydraulic permeability of each well was measured simultaneously with DI water. The membranes were then modified by adding monomer solution (200 μL) to each well, shaking the plates on an orbital shaker at 100 rpm for 1 hr, reducing O_2 level under vacuum for 10 min (or purging with N_2 for 15 min), and irradiating plates in the UV chamber for 30 s (unless otherwise noted). After modification, the plates were washed by shaking in water for 1 h.

Each 96-well plate allowed evaluation of 22 monomers with four replicates for each monomer, and eight controls: four membrane coupons were treated with ethanol without UV irradiation to serve as a control for membranes grafted with the monomers dissolved in ethanol, and the remaining four wells were used as-received to serve as a control for the membranes grafted with monomers dissolved in water.

Evaluation of Modified Membranes by Filtration. The resistance of the 96 membrane coupons during protein solution filtration was measured simultaneously by mounting the filter plate on a vacuum manifold (Pall Corporation, East Hills, NY) as shown schematically in Figure 3. A constant transmembrane pressure (TMP) of 9.8 psi was provided by a vacuum pump. First, water and then PBS solution flux were measured, and then 300 μL of foulant solution was added to each well. A vacuum was then applied for 4 min., after which the wells were gently emptied and refilled with PBS solution. The PBS flux was measured again, followed by a DI water measurement.

During filtration, a 96-well receiver plate was placed under the 96-well filter plate. The permeate from each membrane was collected into a corresponding well in the receiver plate, and was analyzed for solute concentration and volume (as described later) to calculate the rejection and flux properties of each membrane. The receiver plate was replaced by an empty receiver plate every time the wells of the membrane plate were refilled.

Evaluation of Modified Membranes by Static Protein Adsorption. The resistance of modified and control membranes was also evaluated using a static adsorption protocol. The water permeability after adsorption was measured as a criterion for membrane performance. In this method, 300 μL of protein solution was added to each well, and the plate was sealed with adhesive film to eliminate evaporation. The plate was then placed on a shaker (as above) for 44 h. After equilibration, the wells were then gently emptied, and DI flux was measured. The membrane resistance was calculated using flux values. The resistance increase of the modified membranes caused by protein adsorption was compared with that of control membranes to evaluate protein/surface interactions.

Analytical Methods. A Microplate Spectrophotometer (PowerWave XS, BioTek Instruments Inc., Winooski, VT), was used to measure the volume and solute concentration of permeate solution in the receiver plate wells. The acrylic 96-well receiver plates are UV transparent which facilitates permeate analysis by light absorbance in both UV and near infrared regions. Protein concentrations were measured at 280 nm. Rejection, $\mathcal{R}$, was calculated as $\mathcal{R} = 1 - C_p/C_f$, where C_p (g L^{-1}) and C_f (g L^{-1}) are the concentrations in the permeate and feed, respectively. The volume of permeate in each receiver well was measured at 977 nm. Proteins do not absorb at this wavelength, whereas water exhibits an absorbance peak. Volumetric flux, J_v (m s^{-1}) was calculated as $J_v = V/At$, where V (m^3) is the cumulative permeate volume, A (m^2) is the membrane area, and t (s) is the filtration time. The resistance of membrane was calculated from $R = \Delta P/\mu J_v$, where ΔP (Pa) is the transmembrane pressure, μ (g m^{-1} s^{-1}) is the solution viscosity at 22 ± 1°C.

Validation of the HTP-PGP method

The HTP-PGP approach was validated by comparing results with those obtained from a conventional bench-scale (25 mm stirred cell) low-throughput approach using the six monomers (NVP, HEMA, AA, AAG, SPMA and AMPS) described by Taniguchi and Belfort.[8] For each monomer, five concentrations (0.02, 0.05, 0.1, 0.2, and 0.5 mol L^{-1})

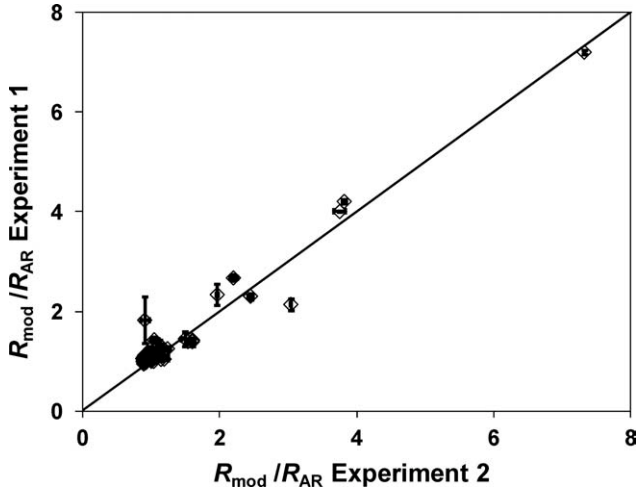

Figure 5. HT platform reproducibility.

Resistance after modification, R_{mod} normalized by the resistance of the as-received membrane, R_{AR} is plotted for two separate experiments. The correlation coefficient $R^2 = 0.948$, thus verifying reproducibility of the HT platform.

and two UV irradiation times (10 and 30 s) were used for graft polymerization. In the experiment, 12 membrane coupons served as unmodified controls; and another 12 coupons were UV irradiated in the absence of monomers (six coupons were irradiated for 10 s, and another six for 30 s). After modification, the grafted membranes were evaluated by both filtration and static adsorption of BSA. The filtration results were used to compare the HTP-PGP data with published data using the bench-scale approach, which used a filtration assay. The static adsorption experiments were run to assess whether such a protocol was consistent with the filtration assay.

Bench-scale verification methods

Preparation of Modified Membranes. The membranes were modified using the same UV irradiation system as applied in HTP-PGP experiments. The membranes were dipped in monomer solutions for 1 h with a shaking speed of 100 rpm, removed from the monomer solutions, N_2 (water saturated) purged for 15 min (or vacuumed for 10 min), and irradiated for 10 s. After modification, the membranes were washed with DI water by shaking for more than 2 h.

Evaluation of Modified Membranes by Filtration. A dead-end stirred cell filtration system was used to characterize the filtration performance of unmodified and modified membranes. The system consisted of a filtration cell (25-mm diameter, model 8010, Millipore Corp., Bedford, MA) with a total inner volume of 18.5 mL that was connected to a 1-L reservoir. The active membrane area was 3.8 cm^2. The feed side of the system was pressurized with nitrogen. All filtration experiments were conducted at a constant transmembrane pressure at 20 psig, a stirring rate of 500 rpm, and a system temperature of 22 $\pm$ 1°C. The filtration protocol used was based on that described previously[8]; a schematic representation is shown in Supporting Information Figure S1. Each membrane was first compacted for 15 min at 25 psig with DI water. The DI water and PBS permeation resistance (R_M and $R_{M,PBS}$, m^{-1}) was measured. Then, the cell was filled with the BSA solution from the reservoir and the cell repressurized to the operating pressure, 20 psig. The flux was recorded with a time interval of 1 min by a computer, which was connected to an analytical balance. The filtration was stopped after 20 g of permeate was collected. The resistance at this point was called R_F (m^{-1}). The concentration of the feed solution (C_f, g L^{-1}) and the first 2 g of permeate (C_p, g L^{-1}) was measured. Rejection, $\mathcal{R}$, was calculated as $\mathcal{R} = 1 - C_p/C_f$.

Results and Discussion

Validation of HTP-PGP technique

Reproducibility of HTP-PGP Technique. Two separate experiments were conducted with 60 monomers to evaluate the reproducibility of the HT platform. The data from these experiments were analyzed to assess reproducibility in terms of the resistance after modification, R_{mod} (m^{-1}) normalized by the resistance of the as-received membrane, R_{AR} (m^{-1}), shown in Figure 5. The data from two independent experiments are plotted on each axis; the correlation coefficient, $R^2 = 0.948$, indicating excellent correspondence between the experiments.

Comparison of the HTP-PGP and Bench-Scale Approaches. We have validated the HTP-PGP method by comparing measures of membrane performance obtained using the HTP-PGP approach with those of Taniguchi and Belfort,[8] which were obtained using a bench-scale, low-throughput approach. Most of the results obtained in the previous work using the bench-scale method were presented as a function of degree of grafting (a measure of grafted amount) or membrane surface wettability (cosine of the interfacial contact angle).[8] To facilitate comparison, previous results were replotted here as a function of monomer concentration. Figure 6a shows total fouling resistance, ΔR_F ($= R_F - R_{M,PBS}$) as a function of monomer concentration measured using the HTP-PGP approach whereas Figure 6b shows the data reported by Taniguchi and Belfort.[8]

Although the data cannot be compared point-to-point because different monomer concentrations and UV irradiation times were used, the data in Figures 6a,b clearly confirm that the results obtained by the two methods are consistent. Indeed, considering that completely different UV irradiation systems were used, and the base membrane was made by a different manufacturer, the consistency is remarkable. The total fouling obtained by using both methods decreased in the order AAG $\approx$ AA $>$ AMPS $>$ SPMA $>$ HEMA $\approx$ NVP. These comparisons confirm that the HTP-PGP technique identifies the same trends identified using a bench-scale experimental approach and is therefore scalable in terms of membrane resistance developed by interactions between the surface and feed components. As shown in Supporting Information Figures S2 and S3, the HTP-PGP technique is also scalable in terms of membrane resistance after grafting, and protein rejection. A comparison of cleaning efficiency after fouling was not possible because backwashing was used in the bench-scale approach.[8] It should also be

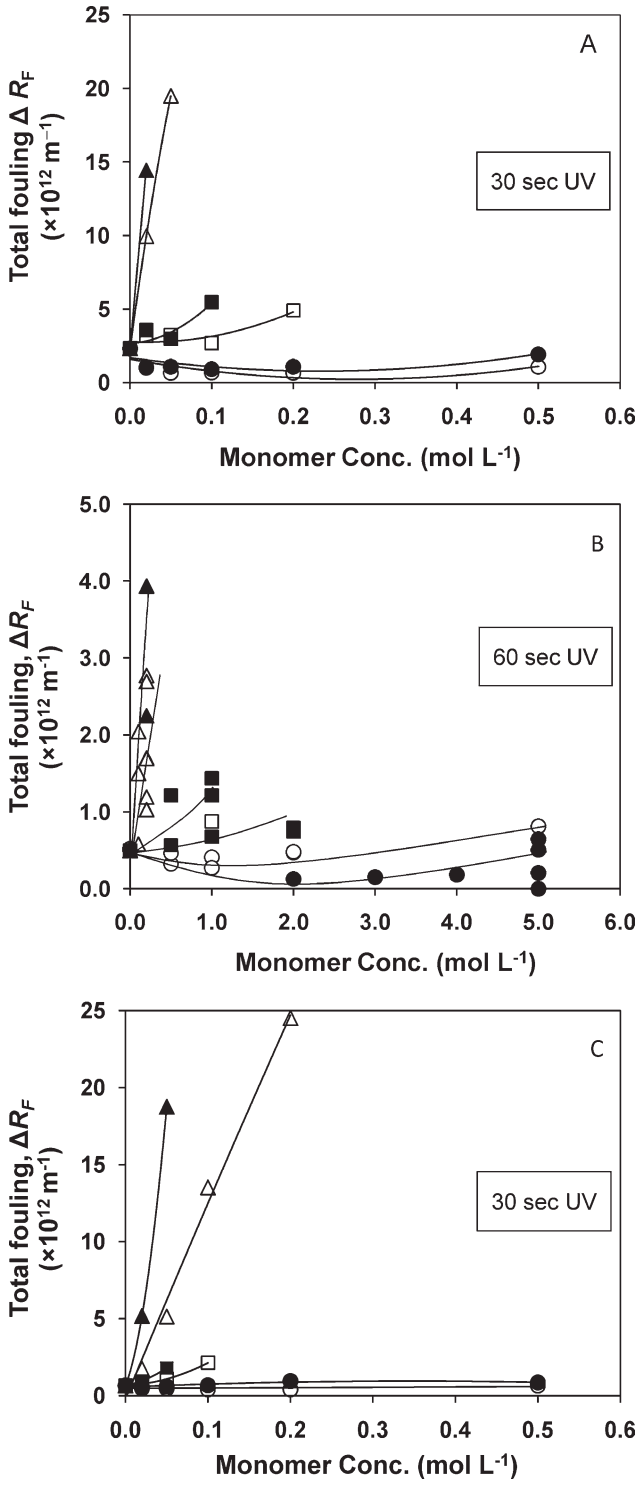

Figure 6. Validation of the "HTP-PGP" technique.

Panels (a) and (b): total fouling, ΔR_F (= $R_F - R_{M,PBS}$) during BSA solution filtration as a function of monomer concentration. Panel (a) shows results obtained using the HTP-PGP approach using an irradiation time of 30 s; (b) shows results from bench-scale data reported by Taniguchi and Belfort[8] using an irradiation time of 60 s. Panel (c): total fouling, ΔR_F (= $R_F - R_{M,PBS}$) during PBS buffer filtration after BSA static adsorption using an irradiation time of 30 s. Monomers: (○) HEMA; (●) NVP; (△) AA; (▲) AAG; (□) SPMA; (■) AMPS.

noted that the relative ranking of different surfaces prepared using the HT approach is not very sensitive to monomer concentration. In general, the monomer concentration should be sufficiently high to produce a degree of grafting that has a measurable influence on surface properties. When using the filtration assay used here, the monomer concentration should not be so high as to block membrane pores to an extent that makes permeability too low to measure.

Comparison of the Filtration and Adsorption Assays. The data in Figures 6a,b showed that the HTP approach was scalable in terms of surface properties assessed using a protein solution filtration protocol. An alternative approach to assess protein/surface interactions is to use a protein adsorption protocol. This approach has the advantage that it avoids complications arising from the convective transport of protein to the membrane surface, which can result in the formation of a protein cake. The adsorption protocol was evaluated by comparing the resistance measured during the protein solution filtration assay (Figure 6a) with the resistance during filtration of a protein-free feed after static adsorption from a protein solution, shown in Figure 6c. Comparison of the data in these figures confirms that the filtration and static adsorption evaluation protocols exhibit similar trends in measured resistance. The general agreement of the results validates the scalability of the static adsorption protocol.

Careful inspection of Figure 6a,c reveals differences between the magnitude of resistance developed during filtration and static adsorption of BSA - the magnitude of resistance was generally greater after filtration as compared to static adsorption. This is expected because resistance was developed by different mechanisms. Pore blockage and pore constriction may result from solute adsorption to the membrane surface and pore walls; the amount depends on binding affinity and the thermodynamic driving force. During filtration, however, permeation drag causes mass accumulation on the membrane surface and in the membrane pores (in proportion to both membrane flux and solute rejection), and may induce protein aggregation; therefore, cake formation may occur in addition to pore blockage and pore constriction. Hence, the resistance after static adsorption better represents the fouling potential of membrane surfaces in terms of solute affinity, chemistry, and structural properties.

HT discovery of new surfaces

The resistance to water permeation after modification (but before fouling) relative to the resistance of the as-received membrane, R_{mod}/R_{AR}, represents the factor by which membrane resistance increased after modification, and is a rough indicator of the amount of grafted material. To assess protein/surface interactions, a fouling index, $\Re$, was calculated as the resistance increase of grafted membranes caused by fouling normalized by that of ungrafted membrane control, $\Re = \Delta R_{mod}/\Delta R_{control}$, where $\Delta R_{mod} = (R_{fouled} - R)_{mod}$ and $\Delta R_{control} = (R_{fouled} - R)_{control}$. The control was the as-received membrane treated with either water or ethanol, depending on which was used to dissolve the monomer. The increase in the modified membrane resistance after protein adsorption should be lower than that of the control when the modified surface resists protein interactions. Although not a general criterion, for applications involving permeation, the

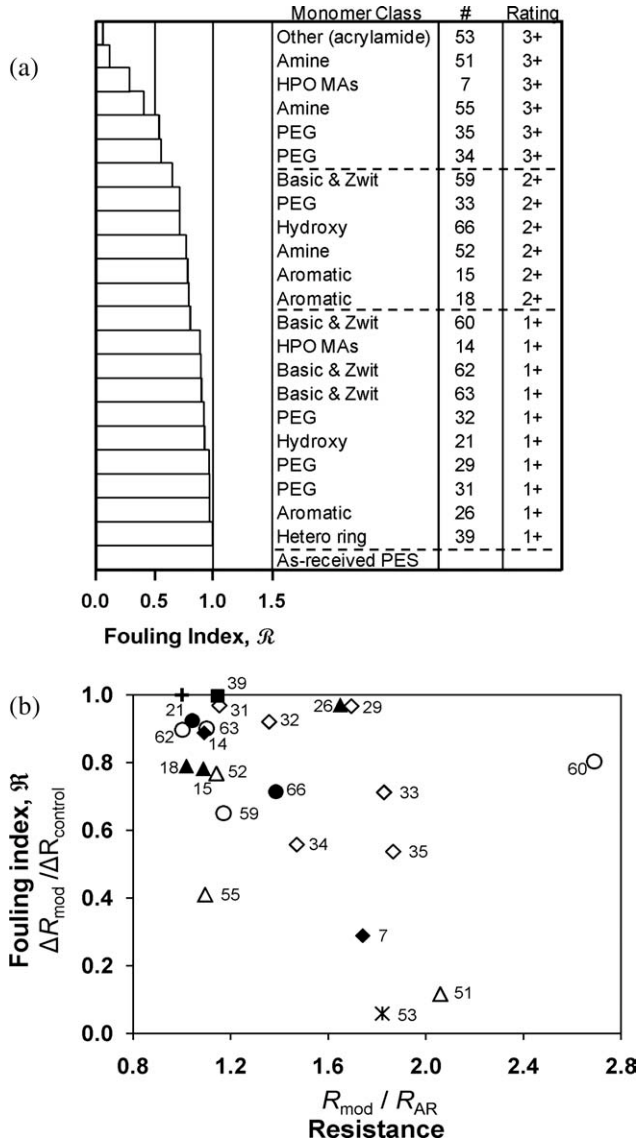

Figure 7. Optimized selection of surfaces for BSA.

A total of 66 commercial monomers were evaluated relative to the as-received poly(ether sulfone) membrane using the HTP-PGP method. Success is measured in terms of a fouling index, $\Re = \Delta R_{mod}/\Delta R_{control} = (R_{fouled} - R)_{mod}/(R_{fouled} - R)_{control}$. Other surfaces exhibit either fouling greater than the as-received membrane, or high membrane resistance. (a): relative ranking of monomers in terms of fouling index; (b): fouling index and increase in resistance to DI water filtration after modification considered together. Symbols are: (◆) Methacrylates having hydrophobic side chains; (■) Hetero ring group monomers; (▲) Aromatic monomers; (●) Hydroxy monomers; (◇) PEG monomers; (□) Acid monomers; (△) Amine monomers; (○) Basic and Zwitterionic monomers; (*) Monomers that cannot be characterized into the other eight classes; (+) Unmodified membrane surface.

membrane resistance for the modified membranes should be near that of the as-received membrane ($R_{mod} \approx R_{AR}$), although a higher resistance may be acceptable when it correlates with increasing rejection (and high rejection is a goal).

Static Adsorption of Protein by Modified Membrane Surfaces and their Subsequent Filtration Resistance. Monomer number, name, structure and fouling index are tabulated in Supporting Information Table S1; the fouling index, calculated as described earlier, was rated into 7 classes as shown by the criteria in Supporting Information Table S2. Representative results are shown in Figure 7. In Figure 7a monomers having a fouling index less than the as-received membrane are ranked. It is clear that the HT approach has identified many new and previously reported surfaces that perform significantly better than the as-received membrane, offering significantly lower resistance due to fouling. In Figure 7b the value of the fouling index is plotted against the resistance after modification. Modification has significantly increased the resistance of some surfaces (e.g., $R_{mod}/R_{AR} \geq 1.8$); however, two such surfaces offer a very low fouling index, i.e., $\Re < 0.1$. Furthermore, many surfaces exhibit a fouling index $\Re < 0.8$ and a relative resistance $R_{mod}/R_{AR} < 1.2$.

The ranking of monomers in terms of the number of monomers with specific ratings for different monomer classes for BSA fouling-resistant surfaces is shown in Figure 8. As expected, on the basis of other results reported in the literature, poly(ethylene glycol) (PEG) surfaces were generally protein resistant, and the fouling index decreased with increasing molecular weight.[22,23] Such selection of previously known protein resistant polymeric surfaces, i.e., those reported in the literature by various research groups, helps build confidence in the HTP-PGP method. Six excellent surfaces (with ratings 3+) were obtained, with two from the PEG class (long-chain poly(ethylene glycol) methyl ether methacrylates, #34 and #35), two from the amine group (*N*-isopropylacrylamide and 2-(dimethylamino) ethyl methacrylate, #51 and #55), one methacrylate having hydrophobic side chains (2-ethylhexyl methacrylate, #7), and diacetone acrylamide (# 53). In addition, good performance was obtained with zwitterion #59, [3-(methacryloylamino) propyl]dimethyl(3-sulfopropyl) ammonium hydroxide inner salt; short chain PEG #33, poly(ethylene glycol) methyl ether methacrylate; and hydroxyl #66, caprolactone 2-(methacryloyloxy) ethyl ester.

The surfaces that exhibited the least favorable fouling index (a rating of 3-) were made using monomers from the acid group and methacrylates having hydrophobic side chains. In addition, no acceptable surfaces (those that exhibited fouling less than the as-received surface) were obtained using monomers containing an acid group unless part of a zwitterionic structure.

Our results are generally consistent with results from studies of protein interactions with surfaces having a variety of functionality created using self-assembled monolayers (SAMs) of alkanethiolates on gold as a model substrate.[22–29] Such studies have identified general features of surfaces having low affinity for proteins: (i) they are hydrophilic (wettable), (ii) they contain hydrogen bond acceptors, (iii) they lack hydrogen bond donors, and (iv) they are electrically neutral.[22,23,25,30] The PEG monomers, representing the "standard" for protein resistance, satisfy all four criteria. However, the best performing monomer, diacetone acrylamide (#53), contains a secondary amine which can act as a hydrogen bond donor (Supporting Information Table S3). It

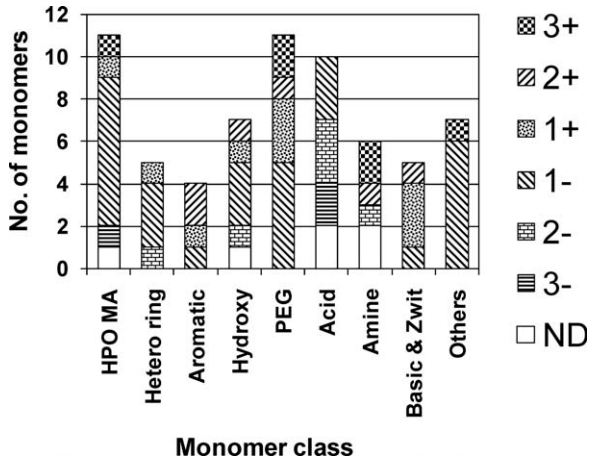

Figure 8. Ranking of monomers by class.

Number of monomers with specific ratings for different monomer classes for fouling-resistant surfaces due to the challenge from BSA. The rating of monomers is according to the fouling index $\Re = \Delta R_{mod}/\Delta R_{control}$: >3 (−−−); 1.8 to 3 (−−); 1 to 1.8 (−); 0.8 to 1 (+); 0.6 to 0.8 (++); 0 to 0.6 (+++).

is likely that the location of the amine group adjacent to the carbonyl oxygen limits its reactivity. Others have noted that primary and secondary amines adsorb more protein than structurally similar groups in the form of amides.[25] Furthermore, it should also be noted that other molecules containing hydrogen bond donors, such as mannitol, have exhibited protein resistance.[31] Two other monomers containing amine groups also performed well. The 2-(dimethylamino) ethyl methacrylate (#55) contains a tertiary amine and the *N*-isopropylacrylamide (#51) also contains an amide group. The caprolactone 2-(methacryloyloxy)ethyl ester monomer (#66) terminates in an alcohol group, separated from the hydrophilic portion of the molecule by a five-carbon chain. It is possible that this chain length is long enough to promote self-association and reduce the hydrogen bond donor reactivity with proteins. The zwitterion [3-(methacryloylamino)propyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt (#59) conforms to the net neutrality criterion, but also contains a secondary amine in an amide group. The most surprising monomer was the 2-ethylhexyl methacrylate (#7), which is much less hydrophilic than the other high performers.

Bench-scale verification of HTP results

Several of the most promising monomers identified in the high throughput experiments (and one that was not among the best) were tested in bench-scale filtration experiments with mixing to assess the scalability of the results and whether surfaces that exhibited low protein interactions after adsorption would also be favorable for filtration applications. Note that the membranes used for the HTP and bench-scale experiments were both made from PES and had a MWCO of 100 kDa. However, the manufacturer of the 96-well plates did not report the membrane source; therefore, it is possible that the membranes used at the two scales were different. Representative filtration data are shown in Figure 9. Data were selected to represent each of the most successful monomer groups identified in the HTP experiments; some data have been omitted for clarity. Also, two of the nine best surfaces that exhibited low protein interaction in the high throughput experiments, monomer #53, diacetone acrylamide and hydroxyl monomer #66, caprolactone 2-(methacryloyloxy)ethyl ester, yielded permeabilities too low to allow evaluation at the bench scale. One monomer that was not among the best candidates, hetero-ring monomer #39, 4-acryloylmorpholine, is also shown. The data are plotted in terms of resistance versus volume throughput; plotting the data in this way minimizes the effects of differences in the initial grafted membrane resistance. As shown in Figure 9, the filtration data for several surfaces clearly show significantly improved performance as compared to the as-received PES membrane (i.e., the lower absolute value of the total fouling resistance and slopes) , as predicted by the high-throughput experimental results; these include surfaces prepared by grafting amine #51, *N*-isopropylacrylamide; methacrylate #7, 2-ethylhexyl methacrylate; PEG #35, poly(ethylene glycol) methyl ether methacrylate ($N = 45$); zwitterion #59, [3-(methacryloylamino) propyl]dimethyl(3-sulfopropyl)ammonium hydroxide inner salt; and hetero-ring #39, 4-acryloylmorpholine.

The data for the resistance of all surfaces determined at the bench scale after about 55 L m^{-2} solution throughput are summarized in Figure 10, where they are also compared with the fouling index determined at the HTP scale. All of the monomers selected from the HTP results and subsequently verified at the bench scale exhibited a fouling index lower than that of the as-received PES surface. The best surface was made from amine monomer #51. The next best performing surface was synthesized from PEG monomer #34. A large group of monomers performed nearly equally at the bench scale, exhibiting a fouling index ranging from 0.4 to 0.55. These include several basic and zwitterionic monomers

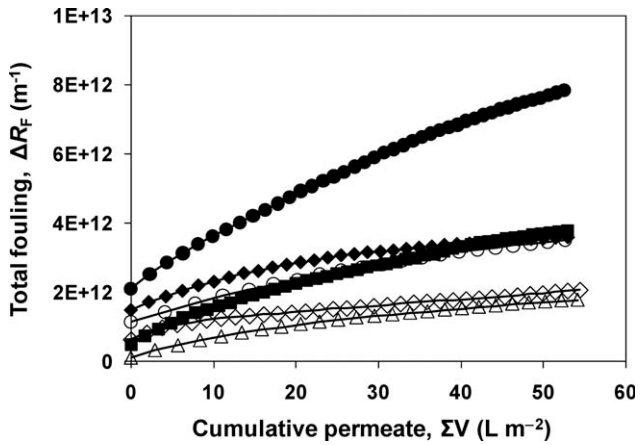

Figure 9. Bench-scale verification of HT discovery.

Total fouling ΔR_F ($= R_F - R_{M,PBS}$) during filtration of BSA in PBS buffer (with mixing) is plotted as a function of cumulative permeate volume throughput. Performance plotted in this way minimizes dependence on initial membrane resistance. All modified surfaces exhibited lower total resistance (membrane resistance + fouling resistance) than that of the as-received membrane. Symbols are: (△) monomer # 51; (◆) monomer # 7; (◇) monomer #34; (○) monomer #59; (■) monomer # 39; (●) Unmodified membrane surface.

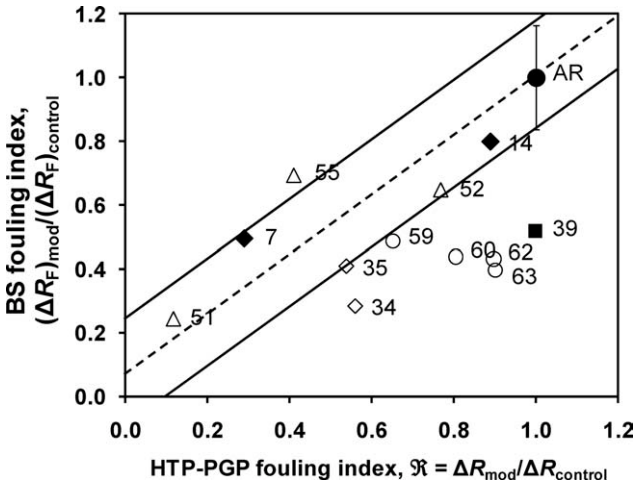

Figure 10. Fouling index comparison at bench- and HT-scale.

Normalized total fouling ($\Delta R_F = R_F - R_{M,PBS}$) during BSA filtration (with mixing) is plotted as a function of fouling index from HTP-PGP experiments. Symbols are: (◆) Methacrylates having hydrophobic side chains; (■) Hetero ring group monomers; (◇) PEG monomers; (△) Amine monomers; (○) Basic and Zwitterionic monomers; (●) Unmodified membrane surface. The lines shown represent one standard deviation based on repeated measurements with the as-received membrane.

(#s 59, 60, 62, and 63), PEG #35, hetero-ring monomer #39, and methacrylate monomer #7; the filtration profiles of several of these monomers are shown in Figure 9.

The fouling index determined from the bench-scale results are compared with those determined from the HTP-PGP results (after static adsorption) in Figure 10. The unmodified membranes were evaluated twice in bench-scale filtration, and the error bar is plotted. Bench scale results correlated well with results from the HT platform: 5 of 12 surfaces were within one standard deviation and 8 of 12 surfaces were within two standard deviations of the 1:1 correspondence line. Five monomers (#s 34, 35, 59, 52, and 14) showed somewhat lower fouling in bench-scale verification whereas three surfaces, those made from monomers (#s 7, 51, and 55), exhibited higher fouling in bench-scale verification than in HTP-PGP screening. The four surfaces that were not within two standard deviations of the 1:1 line, monomers (hetero-ring #39, zwitterionic #60, and basic #s 62 and 63) performed *better* at the bench scale than at the HTP scale.

Despite differences in membrane properties (surface chemistry and resistance), hydrodynamics, and fouling mechanisms, the performance of the monomers at the two different scales correlated reasonably well. The main objective of the current work was to identify surface chemistries that minimize interactions with feed components as a way to mitigate the initial stages of fouling. The resistance after static adsorption likely represents the fouling potential of membrane surfaces in terms of feed component affinity, chemistry, and structure. This approach should incorporate pore blockage and pore constriction that results from solute adsorption to the membrane surface and pore walls. However, this approach neither does incorporate all fouling mechanisms nor does it predict the effects of hydrodynamics. Therefore, minimizing deviations between high throughput and bench scale data will require minimizing the differences in the operating conditions as a function of scale, especially the effects of mixing (absent at the HTP scale) and how the fouling mechanisms were influenced by permeation flux (including effects of concentration polarization).

Discussion and Comparison with Literature

In the following discussion, we divide the monomers studied in this work into four groups: (i) those that have been studied previously but not in the context of either protein interaction or membrane fouling during filtration; (ii) those that have been studied for their properties to resist protein adsorption or cell adhesion but have not been assessed for downstream processing filtration applications; and (iii) those that have been identified previously as promising in filtration applications as a route to mitigate fouling or to enhance recovery. A detailed list of references from the literature for the different monomers is presented in the Supporting Information References. For monomers in group (i), there was no basis in the literature for anticipating how these monomers would perform in terms of protein adsorption or filtration; therefore, our results demonstrate the ability of the HTP to identify new high-performance surfaces and assess them for protein interaction. For monomers in group (ii), our results demonstrate the ability of the HTP to rapidly assess promising surfaces for specific feed solutions. The literature results for monomers in group (iii) serve to validate the HTP approach - the HTP approach identified monomers expected to perform well. In addition, our results illustrate how the HTP approach can be used for verification and optimization.

Group (i). Six monomers studied in this work have appeared in the literature, but not for their surface properties or for applications involving protein adsorption or cell adhesion, or filtration [Group (i)]. These include amide-containing monomer #53, diacetone acrylamide; zwitterionic monomer #63, [2-(methacryloyloxy)ethyl] trimethylammonium methyl sulfate; and monomer #66, caprolactone 2-(methacryloyloxy)ethyl ester. At the HTP scale, these were rated +++, +, and ++ respectively, performing better than the as-received membrane. Monomers #53 and #66 were not subsequently assessed at bench scale because the permeability was too low; these monomers will be investigated further in future work as they could have value for marine, medical and other applications. However, monomer #63 performed quite well at the bench scale; indeed, better than expected based on HTP results.

Aromatic monomer #15, phenyl methacrylate, was cross-linked with divinyl benzene to create novel supports.[32] It performed surprisingly well at the HTP scale, receiving a ++ rating, but was not pursued at the bench scale because the yielded permeability was too low to allow the evaluation. Hetero-ring monomer #39, 4-acryloylmorpholine, has been studied for its metal binding properties as a co-polymer with, e.g., cellulose and 2-acrylamido glycolic acid.[33,34] At the HTP scale, it exhibited reduced interactions with BSA as compared with the as-received membrane, receiving a + rating. Because of its unique structure, it was pursued further at the bench scale, and performed quite well. The metal interaction properties of basic monomer #62, [3-

(methacryloylamino)propyl] trimethylammonium chloride have been studied when used as a component of cross-linked co-polymers.[35,36] Also, copolymers of [(3-methacryloylamino)propyl] trimethylammonium chloride and 2-acrylamido-2-methyl-1-propane sulfonic acid were complexed with Ag(I) and evaluated for their biocidal or biostatic action against *E. coli* and *S. aureus*.[37] In addition, complexes formed between the positively charged random copolymers of methoxy-poly(ethylene glycol) monomethacrylate and [3-(methacryloylamino)propyl] trimethylammonium chloride with oppositely charged biosurfactants (sodium deoxycholate and sodium cholate) were assessed as vectors for drug delivery.[38] In our work, this monomer exhibited reduced interactions with BSA as compared with the as-received membrane at the HTP scale, receiving a + rating, and performed even better at the bench scale. Clearly, the results of Group (i) monomers also demonstrate the ability of the HTP to identify new high-performance surfaces and assess them for protein interaction.

Group (ii). These monomers have been studied for their ability to resist protein adsorption or cell adhesion, but have not been evaluated for their ability to reduce fouling or feed component adhesion during membrane filtration. Star copolymer networks were synthesized by the group transfer polymerization of aromatic monomer #18, benzyl methacrylate, and the ionizable hydrophilic monomer 2-(dimethylamino)ethyl methacrylate (DMAEMA) were cross-linked by ethylene glycol dimethacrylate.[39] DNA adsorbed strongly onto the networks at low pH but there was no DNA adsorption at alkaline pH (pH $>$ 10), when the DMAEMA units were uncharged, indicating that DNA binding was governed by electrostatics.[39] In our work, benzyl methacrylate performed surprisingly well at the HTP scale, receiving a ++ rating. It was not among the top performers, however, and was not pursued further at the bench scale, in part due to its hydrophobic structure. Surfaces grafted with *N*-isopropylacrylamide (#51) have exhibited protein adsorption properties based on its temperature-dependent conformation, and, therefore, could be used to control protein and cell adhesion/detachment behavior.[40–47] At temperatures above its lower critical solution temperature (32°C),[43] polymer chains were collapsed and protein adsorbing whereas below this temperature they were hydrated and protein repellent.[41] In our work, this monomer created one of the best surfaces at the HTP scale, and was the best performer at the bench scale. Future work with this monomer will involve optimization and assessment of its pH, salt, and thermoresponsive properties.

The brushes formed by 2-(dimethylamino)ethyl methacrylate (#55) have been shown to be sensitive to solution pH and ionic strength; increasing either leads to a conformation switch from a stretched brush to a collapsed state. In contrast to polymerized *N*-isopropylacrylamide, the collapsed state enhances hydrophilicity and protein-resistance of the grafted surfaces, due to a higher surface enrichment of ester groups.[48–51] A weak polyelectrolyte ultrafiltration membrane based on poly(acrylonitrile and 2-dimethylamino ethyl methacrylate) copolymer was reported.[50] X-ray photoelectron spectroscopy confirmed enrichment of PDMAEMA on the membrane surface, which made water flux was tunable by switching from the stretched to collapsed state. Surface enrichment of ester groups improved water flux[50]; however, effects on fouling were not examined. In our work, this monomer performed quite well at the HTP scale, as one of the top performers. However, although it did perform significantly better than the as-received membrane at the bench scale, it was not one of the top performers. Future work will involve optimizing solution conditions to assure that a favorable conformational state has been achieved. Monomer *N*-tert-butylacrylamide (#52) has been used to form thermoresponsive surfaces to control bioadhesion of protein and bacterial, and cell attachment and growth.[52–55]

Zwitterionic surfaces have been investigated as protein resistance surfaces based on cell surface lipids as biological precedents and the previous success of grafted phosphocholine derivatives.[22] Surfaces comprised of single component thiols terminated in groups having both a positively charged moiety (quaternary ammonium) and a negatively charged moiety (either phosphate or sulfonate) resisted adsorption of fibrinogen and lysozyme. In addition, surfaces comprised of 1:1 mixtures of thiols terminated in a positively charged group (quaternary ammonium) and a negatively charged sulfonate group also resisted protein adsorption.[22] Cho et al.[56] showed that the surface grafted with zwitterionic monomer #59 exhibited resistance to the nonspecific adsorption of proteins, comparable to that of the best known systems such as PEG-like films. In our work, at the HTP scale this monomer performed well, receiving a ++ rating, and it performed similarly well at the bench scale.

Group (iii). These monomers have been studied for their ability to resist protein adsorption or cell adhesion during membrane filtration. To our knowledge, only a few of the monomers studied in this work fall into this category. One monomer considered here, 2-ethylhexyl methacrylate (#7), was used as a co-polymer additive to segmented polyurethane to improve its biocompatibility with blood.[57–63] The co-polymer was made from 2-ethylhexyl methacrylate and a phospholipid polymer having 2-methacryloyloxyethyl phosphorylcholine units. Membranes made from this "alloy" were evaluated for insulin permeation and fibroblast adhesion. A reduction in adhesion was promoted by the co-polymer additive, which was dispersed both on the surface and inside the membrane. In our work at the HTP scale this monomer performed very well, receiving an excellent (+++) rating, and it performed similarly well at the bench scale. A random copolymer was synthesized from acrylonitrile and amine monomer #55, poly((2-dimethylamino ethyl methacrylate)-acrylonitrile) and then reacted with 1,3-propane sultone to produce [2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide moieties (monomer #60) on the surface.[51] As a result, the hydrophilicity, antifouling properties, and flux recovery after water cleaning were improved. Recently, Azzaroni et al.[64] have studied graft polymerization of sulfobetaines (i.e., zwitterionic monomer #60) on silicon and gold surfaces to form polymer brushes. Thin brushes were wettable and swelled in water due to strong hydration forces and because steric factors (related to the polymer backbone and the hydrated charged groups) hindered the formation of ion pairs. As the brush thickness increased, a decrease in the dielectric constant of water in the polymer environment and a high polymer volume fraction promoted ion paring, leading to a hydrophobic,

self-associated collapsed state that could be partially reversed by increasing the temperature to about 50°C. The good performance of this monomer in our work suggests that the surface conformation is in a nonassociated and hence highly hydrated state.

Many researchers have observed that poly(ethylene glycol) surfaces resist nonspecific adsorption of proteins; this property has resulted in their wide use in biomedical devices.[65,66] Protein resistance has been observed to increase with density and chain length of surface grafted PEGs, a finding that is consistent with the results of this work.[22] Self-assembled monolayers terminated in $(EG)_nOH$ groups have also been shown to resist protein adsorption; because of this property and because they are well characterized, they are considered the standard against which to compare other systems.[23] Potential limitations of PEGs include their propensity to degrade both in the presence of dioxygen and transition metal ions, and their inability to retain anti-fouling properties above 35°C.[31,67–69] Still, the favorable properties of PEGs have motivated attempts to use them to improve membrane surface chemistry. Microfiltration (MF) membranes were fabricated by phase inversion in aqueous media from comb copolymers synthesized via living radical graft polymerization of poly(ethylene glycol) methyl ether methacrylate (monomer #30–35) with poly(vinylidene fluoride) (PVDF) in the reversible addition fragmentation chain transfer-mediated process. The copolymer MF membranes displayed resistance to γ-globulin fouling, in comparison to the unmodified hydrophobic PVDF MF membranes.[70] A similar approach was taken to make thin film composite membranes.[71] The surface was coated with amphiphilic graft copolymers consisting of a PVDF backbone and poly(oxyethylene methacrylate) side chains. These materials molecularly self-assemble into bicontinuous nanophase domains of semicrystalline PVDF and poly(ethylene oxide), providing selective transport channels of defined size. The ability of such membranes to resist fouling was attributed to hydrogen bonding between water and the ether oxygen groups of the PEO side chains, which act as a grafted brush layer at the membrane surface and within the water-filled nanochannels. In other research, poly(poly(ethyleneglycol) methyl ether methacrylate) was covalently grafted onto UV pretreated PVDF microfiltration membrane surfaces by reverse atom transfer radical polymerization.[72] Protein adsorption and permeation flux experiments revealed that the grafted exhibited antifouling properties as compared with ungrafted PVDF.[72] In our work, this monomer performed quite well at the HTP scale, as one of the top performers, and it performed equally well at the bench scale. These results provide verification for the HTP approach—it was able to identify surfaces that are known to resist protein fouling.

Conclusions

A novel high throughput method for synthesis and screening of customized protein-resistant surfaces was developed by combining a high throughput platform approach together with our patented photo-induced graft polymerization method, to allow facile modification of commercial poly(aryl sulfone) membranes. The following conclusions can be made from the development and testing of this new method of synthesis and screening:

- The PGP method combined with a HTP approach is an inexpensive, fast, simple, verifiable, reproducible, and scalable method for identifying surfaces that exhibit low protein adsorption and hence low fouling during membrane filtration.
- The HTP approach was used in a discovery mode to identify many surfaces that perform significantly better than the as-received membrane, offering significantly lower resistance due to fouling and similar membrane resistance. As expected, long chain poly(ethylene glycol) surfaces were generally protein resistant. Excellent surfaces were also obtained with two monomers from the amine group (*N*-isopropylacrylamide and 2-(dimethylamino) ethyl methacrylate), one methacrylate having hydrophobic side chains (2-ethylhexyl methacrylate), and diacetone acrylamide. The surfaces which exhibit the least favorable fouling index were made using monomers from the acid group and methacrylates having hydrophobic side chains. In addition, no acceptable surfaces (those that exhibited fouling less than the as-received surface) were obtained using monomers containing an acid group unless part of a zwitterionic structure.
- Despite significant past efforts, a clear understanding of the mechanism of how substrate surface chemistry influences protein behavior is lacking. Guidelines for designing protein-resistant surfaces have emerged from studies of protein interactions with functionalized self-assembled monolayers as model substrates. They include increased hydrophilic character (i.e., high wettability), presence of a large number of hydrogen bond acceptors, absence of hydrogen bond donors, and the need for electrical neutrality. Polyethylene glycol and zwitterionic surfaces have received special attention, as they appear to repel proteins efficiently. Our results are generally consistent with these guidelines and may, with further analysis, provide more insight regarding mechanism.
- Because of the high throughput nature of the HTP-PGP method, the volume of feed and the duration of the tests were significantly lower than those for similar past laboratory tests. Thus, the HTP approach is about six times faster (quadruplicate runs) than the previous bench-scale study (duplicate runs), and the identification of new protein resistant surfaces from a screened library of 66 commercial vinyl monomers took only about 6 weeks for one protein-containing feed.

Acknowledgments

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Notation

$\mathcal{R}$ = solute rejection
C_p = solute concentration in the permeate, g L^{-1}
C_f = solute concentration in the feed, g L^{-1}
J_v = volumetric flux, m s^{-1}
V = cumulative permeate volume, m^3
A = membrane active area, m^2

t = the filtration time, s
R = resistance of membrane, m^{-1}
ΔP = transmembrane pressure, Pa
μ = viscosity of the solution at 22 ± 1°C, g m^{-1} s^{-1}
R_M = membrane resistance to DI water, m^{-1}
$R_{M,PBS}$ = membrane resistance to PBS buffer, m^{-1}
R_F = membrane resistance to BSA solution at the end of filtration, m^{-1}
R_{mod} = resistance of modified membrane to DI water, m^{-1}
R_{AR} = resistance of the as-received membrane to DI water, m^{-1}
ΔR_F = total fouling, or resistance increase due to fouling ($R_F - R_{M,PBS}$), m^{-1}
R_{fouled} = membrane resistance to DI water after BSA adsorption, m^{-1}
$\Re$ = fouling index ($\Delta R_{mod}/\Delta R_{control}$)
ΔR_{mod} = resistance increase of modified membrane after adsorption, m^{-1}
$\Delta R_{control}$ = resistance increase of control membrane after adsorption, m^{-1}

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