SURFACE MODIFICATION OF SILICON PHOTONIC MICRORING RESONATORS FOR CHEMICAL SENSING

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DISSESTATION
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ABSTRACT

Silicon photonic microring resonators have emerged as a promising technology for the sensitive detection of biological macromolecules, including proteins and nucleic acids. These bulk property detectors rely on the changes in the effective refractive index of the sensing region, eliminating the need for chromophoric or fluorescently-tagged samples. This robust and versatile sensing platform has sensitivities down to $10^{-7}$ RIU and a linear dynamic range on the order of 1 RIU, eliciting interest in non-biological analytical challenges such as the detection of high molecular weight polymers within gradient separations (currently impossible with the linear dynamic range of differential RI detectors) and the detection of small, non-specifically binding organics, especially toxic and regulated species such as pesticides and carcinogens in real time and at low concentrations.

Functionalizing the surface of silicon photonic microring resonators with covalently bound organics is one detection strategy to both increase detector sensitivity (by localizing the analyte within the organic layer) and lend a degree of selectivity in the partitioning behavior of the analyte into the sensing region (by matching chemistries between the organic layer and the analyte). There are many chemistries compatible with silicon dioxide surfaces, but the two presented within, hydrogels and polymer brushes, lend the researcher unique control over the platform’s chemical and physical properties. Hydrogels, in particular poly(acrylamide) and poly(acrylic acid), have well-defined syntheses and modification routes as they are widely used in pharmaceuticals and agriculture. Patterned enthalpic gradients embedded in hydrogels, for example, have already been used to direct chemical agents across surfaces without the need for external energy input. This surface-directed transport can be used to separate and concentrate analytes directly to the sensor, a critical need as sensor area decreases to the nanoscale.
Interfacing this technology with a microring resonator array would allow for the robust detection of such transported analytes, which are currently limited to those with fluorescent tags.

Surface-initiated polymerization has been used for many years to selectively alter the surface properties, and with the development of atom-transfer radical polymerization as a commonly-used and highly-controlled polymer brush growth method, the researcher has tremendous control over the surface functionalization, allowing for patterning and gradients in chemical and physical brush properties. This is ideal for preparing thin, well-defined organic coatings over the silicon resonators, allowing for rapid diffusion to the sensor surface and even partitioning, while also allowing the researcher to embed specificity in the brush-analyte interactions (Q-poly(2-methacryloyloxyethyltrimethyl ammonium fluoride brushes for detection of coumaphos degradation, for example).

Here a general method for modifying silicon microring resonator arrays with hydrogels and polymer brushes is presented, in addition to an overview of the fundamental processes which can be probed with such modifications. Tuning sensor selectivity and specificity by optimizing interactions between the agent(s) of interest and the polymer construct can lead to response enhancements in excess of 1000% percent, relative to non-functionalized sensors, an important advance in the detection of toxic species such as organophosphates. The combination of microring resonators with recent advances in the creation of precisely controlled gradients within polymeric surfaces might allow for the active and directed transport of concentrated analytes onto specific sensor elements, thereby integrating together the often disparate steps of separation, concentration, and detection to a single sensing device.
To Dr. Henry K. Hall Jr.
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Chapter 1: Introduction

1.1 Project Motivation

The sensitive, selective, and quantitative real-time measurement of non-chromophoric, non-fluorogenic species remains an important challenge for a range of analytical applications, including environmental analysis, consumer safety, and chemical warfare agent detection. Intriguingly, this is not limited to a particular molecular weight range or chemical functionality: high molecular weight polyolefins can be just as difficult to analyze in real time as the small molecule organophosphate sarin. The overarching unifier is that such analyses are complicated by targets which do not contain convenient spectroscopic signatures amenable to simple measurements, thus often requiring more sophisticated detection approaches.

These problems are globally relevant; high molecular weight polymers (~10-100 kDa) find use in a myriad of industrial applications including space shuttle hulls and water bottles, with over 80 million tonnes of poly(ethylene) alone produced annually. Characterization of both polymer molecular weight and polydispersity (in both molecular weight and structure) is critical as these contribute greatly to the chemical and mechanical properties, thereby dictating material use. Characterizing a two dimensional distribution (separating by both molecular weight and branching structure, for example) is hardly straightforward since at least two, preferably orthogonal, separation methods must be coupled together with a compatible detection technique. The most commonly used approach interfaces high performance liquid chromatography (HPLC), which separates based on composition, with size exclusion chromatography (SEC), which separates based on size/hydrodynamic radius. While this coupling usually provides successful separations, finding an appropriate detector to then characterize the isolated samples is difficult considering the diluted samples, high flow rates, and graded solvent composition.
necessary for separation. Because of this, pairing with common spectroscopic (troubled by dilution and lack of unique spectroscopic signature), mass (troubled by molecular weights above ~10^5 Da), or charge detectors often fails.

On the small molecule front, several toxic and regulated species, including but not limited to mutagens, carcinogens, and neurotoxins, continues to be a problem in several industries, especially when considering the low concentrations and complex matrices in which these detections must occur. Bisphenol A, for example, is a regulated, endocrine-disrupting, non-biodegradable small molecule which has potential reproductive implications. Considering its wide use in polycarbonates and epoxies\textsuperscript{10}, it is not surprising that there is an ongoing effort to detect and remediate its presence in wastewater treatment plants.\textsuperscript{11} Carcinogenic plasticizers such as nitrobenzene, methyl isobutyl ketone, and diethyl phthalate have also been used for decades, incorporated into lacquers, detergent, paints, and polishes. The off-gassing of these chemicals over time has been of increasing concern as the long term toxicity is not well understood. Organophosphate-based pesticides are a well-popularized issue of today’s agricultural economics,\textsuperscript{12} with deep-rooted concerns in the neurological damage and permanent physical effects in both animals and humans, children especially, being of top concern.\textsuperscript{13}

Taking this one step further, with recent and repeated sarin attacks in Syria, the detection of chemical warfare agents (CWAs) remains a top priority in defense. CWAs suffer from related detection issues,\textsuperscript{1} a pressing concern considering organophosphate CWAs have IC\textsubscript{50} values on the order of parts per billion.\textsuperscript{14} The molecular architectures of weapons such as sarin, soman, VX, or paraoxan typically lack chromophoric or fluorogenic signatures, which exclude their detection using standard instrumentation such as UV-Vis and fluorescence spectroscopy. Other common analytical techniques such as nuclear magnetic resonance or mass spectrometry require
sample separation and concentration, in addition to interpretation by a skilled operator, none of which is feasible for field deployment. There are field deployable IR, enzymatic panels,\textsuperscript{15} and ion mobility detectors, though these generally require interfacing with filtering or separation technology of some sort, and of course there are several rough colorimetric panels for rapid detection, but many are skewed in the favor of false positives (as opposed to false negatives), a critical but expensive safety margin. While it is undoubtedly better to be safe than sorry when it comes to national security, it is best of all to have superior detectors which are more sensitive and selective towards the analytes.

1.2 Physical Property Detectors and Microring Resonators

Physical property detectors, such as charged aerosol detectors, evaporative light scattering detections, quartz crystal microbalances, and optical sensors are an attractive solution to these detection problems as they do not rely on analyte chromophoric properties, lending them high versatility. Optical sensors, encompassing refractive index sensors such as surface plasmon resonance detectors, interferometric techniques, photonic crystals, and microcavity resonators, have been steadily emerging the preferred bulk-property detectors for this problem.\textsuperscript{16} In particular, silicon photonic microcavities show great promise, owing to their high sensitivity and quality (Q) factor, inherent scalability, and ease of fabrication.\textsuperscript{17} While there are many intriguing architectures being investigated right now, including microtoroids and optofluidic ring resonators,\textsuperscript{18} microring resonators have demonstrated a convenient cross section of cost, sensitivity, and ease of use. See Figure 1.1 for a depiction of the instrumentation.
Figure 1.1: Genalyte Maverick microring resonator platform

A). Representative layout of a microring resonator chip, with an SEM image of a single ring (scale bar 10 µm) and an optical micrograph (credit: Daniel A. McCurry) with a penny for scale.

B). Three of the Maverick instruments on a laser table. Roughly “mini-fridge” size, the size of the instruments could be further reduced. These instruments can be linked to one another (daisy-chain) such that only one laser source is required, and recent instrument iterations for biological applications involve arrays of chips, furthering multiplexing capabilities.

This technology is being commercialized by Genalyte, Inc. as the Maverick detection platform. In the current configuration each sensor array chip is 4 x 6 mm in size and features 132 individually-addressable, 30 µm-diameter sensors. The entire chip is coated with a fluoropolymer cladding layer and selectively removed to expose only 128 of the rings to solution. The remaining sensors can be used to correct for thermal drift, a critical failing in most differential refractometers. These devices are fabricated on silicon-on-insulator wafers at a commercial silicon foundry using standard deep UV photolithography. High fidelity fabrication leads to high Q-factor cavities, which leads to a dramatic increase in the effective optical path length and sharpening of the resonance to an extremely narrow spectral dispersion (FWHM ≈ 50
picometers). Solutions are flowed across the sensor chip via an automated fluid handling system that delivers solution through a laser-cut Mylar gasket, which defines at least two channels per sensor array chip.

Figure 1.2: Signal transduction mechanism

A). Resonance condition has not been met; light cannot couple onto the ring resonator, and instead is transmitted down the waveguide without interference until reaching the output grating coupler and being detected by the photodiode array. The resonance condition is dictated by the effective refractive index of the ring resonator plus part of the surrounding medium.

B). Resonance condition has been met, and incoming light is coupled onto the ring resonator, resulting in a dip in transmission. Light is confined into the resonator via total internal reflection and interacts with the environment through an exponentially-decaying optical profile that has a 1/e decay length of 63 nm.
A tunable external cavity diode laser centered at 1560 nm is coupled on-chip via grating couplers, and the light propagates down a linear waveguide located adjacent to the microring structure. Each ring with has its own set of grating couplers and linear waveguide such that each ring can be interrogated individually. All optical interfaces are done in the far field with light coupled from free-space into and off of the chip from the laser and then to a detection photodiode. The laser is focused onto the input grating coupler of a single waveguide and swept through a 60 nm spectral bandwidth. This architecture supports specific resonant wavelengths, which are governed by the following equation (Eq 1):

\[ m\lambda = 2\pi r n_{eff} \]  

(Eq 1)

where \( m \) is an integer, \( \lambda \) is the wavelength of light, \( r \) is the radius of the resonator, and \( n_{eff} \) is the effective refractive index. The resonant wavelength of this microcavity is highly responsive to changes in the \( n_{eff} \) of the ring, which is dictated by the surface conditions sampled by the evanescent electromagnetic field of the confined light. As the refractive index near the resonator changes, owing to the presence of a new analyte or changing of solvent, the resonance wavelength of modes supported by the cavity is altered and detected as dips in the optical power transmitted through the coupling waveguide past the microring sensor. (See Figure 1.2).

1.3 Interfacing Microring Resonators with other Instrumentation

Previously, this technology was interfaced with HPLC and used to detect small molecules.\(^{19}\) Using HPLC, a solution of ibuprofen and simvastatin were separated and each individual species was detected by the microring resonator platform as it eluted off column (Figure 1.3, adapted with permission from Reference 19, copyright American Chemical Society, 2014).
A). The eluents from an HPLC can be detected as they elute off the column; though diluted from the separation, each analyte can still be detected.

B). An example of real-time resonance shifts of the separation of ibuprofen and simvastatin.

The bulk RI shift as the solvent graded from water to acetonitrile was observed, but the analyte peaks were clearly distinguishable from this bulk change, a feat impossible for conventional RI detectors (limited in the linear dynamic range to 500-600 µRIU, such detectors would not be able to discern any analytes against the background of changing solvent; the MRR platform has a theoretical linear dynamic range of almost 1 RIU!). This proof-of-concept study confirmed the thermal stability and large dynamic range of the microring platform, but did not address how to maximize the sensitivity of the platform to the species of interest. There is significant room for improvement in enhancing the specificity of interactions between the analyte and the sensing region (i.e. evanescent field) by using surface modifications.20
1.4 Surface Modification

Sensitivity and molecular selectivity can be enhanced by rationally engineering the sensor surface chemistry to encourage selective interactions biased towards species of interest. Though not all analytes have specific recognition elements\(^{21,22}\) (i.e. antibodies or DNA compliments), it is possible to take advantage of non-specific chemical interactions such as van der Waals, hydrogen bonding, and electrostatics for enhancing target-specific interactions. Furthermore, gradients of surface functionality could function as another separation step (i.e. analyte partitioning kinetics with differentially functionalized rings will give chemical information for eluents from a SEC column) or as a way to concentrate low abundant species onto a particular sensor. However, for these proposals to be realized, highly controlled sensor surface functionalization methods need to be developed to fabricate reproducible surfaces with specific functionality.
A). “(a) Scanning electron micrograph of an individual microring and adjacent linear waveguide on a silicon-on-insulator chip, exposed to solution through an annular opening in a fluoropolymer cladding layer. (b) Surface polymerization is performed by first functionalizing the rings with initiator followed by exposure to the monomer/catalyst solution. (c) Resonant optical modes supported on the microring are extremely sensitive to changes in the local dielectric environment, shifting to longer wavelengths as polymerization occurs. (d) The shifts in resonance wavelength can be monitored in real time, allowing relative rates of surface-initiated polymerization to be directly observed.”

B). Depiction of the p(SBMA) polymer brush being grown directly off the surface-tethered initiator (BMPOUTS).

Prior work by the Bailey group\textsuperscript{23} has utilized surface-initiated atom-transfer radical polymerization to chemically modify the resonator rings; poly(sulfobetaine methacrylate) was grown directly off the ring resonators, and polymer growth was monitored in real time (Figure
Controlled radical polymerization occurs up to two orders of magnitude slower than free radical polymerization, resulting in precise and low polydispersity polymers. Coupled with real time monitoring of surface growth, something previously impossible to capture, it is straightforward to establish polymer kinetics, which could be valuable when growing novel brushes.

In addition to polymer brushes, highly swollen, environmentally sensitive hydrogels are also of interest as they are easily modified to include transport gradients. This has been seized upon by the nanosensor community in general, which cannot rely on diffusion-driven detection systems in which they are limited by analyte transport, rather than sensitivity. Directed transport of analyte molecules to a nanosensor location lowers limit of detection and speeds detection rate, important in field-operated sensors. Thus far, poly(acrylamide) hydrogels have been modified with embedded enthalpic gradients, which can transport fluorescent materials several millimeters to a particular sensing region\textsuperscript{24} (Figure 1.5, adapted with permission from Reference 24, copyright American Chemical Society 2015).
Figure 1.5: Chemically-modified poly(acrylamide) hydrogels transport analytes across surfaces

A). A depiction of a hydrogel modified with a cationic gradient being dosed with pyranine, the directed transport of which towards the cationic side can be tracked fluorescently.

B). Cartoon of the change in enthalpy across a gradient; this can be used to separate and concentrate different analytes on one platform.

This has great potential applicability if integrated with the microring resonator platform; considering the gels can contain orthogonal gradients, one could envision dosing a microchip with several analytes and allowing the chemical gradients to direct and concentrate different
analytes to individual ring clusters, truly taking advantage of the multiplexable nature of the platform.

1.5 Integration of Polymer Modifications on Microring Resonators

Silicon photonic microring sensor array technology has previously been utilized for the detection of biomolecular targets, including proteins,\textsuperscript{25} miRNA,\textsuperscript{26} and DNA.\textsuperscript{27} This technology has also been applied to monitor layer-by-layer assembly\textsuperscript{20} and chemical reactions occurring at the sensor surface.\textsuperscript{28} As mentioned, when there are no specific binding motif/recognition elements (i.e. antibodies or DNA compliments), detection capabilities significantly decrease, as there is no interaction to localize the analyte within the surface-confined sensing region. The modification of these microring resonator arrays with polymer brushes and hydrogels was attempted to encourage localization of the analytes within the sensing region, enhancing the signal compared to an unmodified ring resonator and allowing for detection of otherwise highly dilute and non-chromophoric species.

First, growing polymer brushes off the ring surface using surface-initiated atom transfer radical polymerization had to be standardized and characterized. Then the polymer brushes were exposed to several analytical standards, including caffeine and acetaminophen, to determine the extent of signal enhancement. Once signal enhancement was established, polymer brush dynamics, such as dissolution and hysteresis, were investigated to test the robustness of the platform. Finally, amorphous hydrogels were tested as a secondary materials class, considering the ease of synthesis and chemical modification routes. Organic modification of the ring resonator platform was shown to enhance sensitivity to certain analytes up to 1000%, and
yielded a great deal of information regarding polymer brush dynamics and how these can be capitalized to maximize sensor sensitivity and selectivity to analytes of interest.
Chapter 2: A Unified Approach to Surface-Initiated Atom-Transfer Radical Polymerization (SI-ATRP)

Notes and acknowledgements: This chapter has been contributed in content and data equally by ALDS and Kali A. Serrano. Dr. Lydia Kisley and Nathan W. Reed are both acknowledged for their assistance in conducting SI-ATRP experiments.

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2.1 Controlled Radical Polymerizations

As a diffusion-controlled reaction, radical polymerizations are completed rapidly (on the order of seconds to minutes) and lack structural control; most polymerizations complete with high polydispersity index from early and unwanted terminations, in addition to a variety of structural variations. While powerful in speed and simplicity, the lack of control in free radical polymerization leaves much to be desired in functional polymeric materials.

Polymerizations which do not permanently terminate upon interruption of growth are known as living polymerizations, and have been increasingly popular for accessing complex architectures and high molecular weights. Also known as “controlled” radical polymerizations, these reactions are marked by a slow propagation and tight polydispersity, valuable in many sensitive applications such as adhesives or responsive surfaces, which are less tolerant to the high polydispersity of free radical polymerizations. Atom-transfer radical polymerization (ATRP) has been established as one of the most versatile controlled radical polymerization approaches currently known. ATRP relies on the dynamic equilibrium between a highly favored,
deactivated state and an unfavorable propagating radical state (P˙), which controls the rate of polymerization (RP) according to the equation (Eq 2):

\[ R_P = -\frac{d[M]}{dt} = k[P^\cdot ][M] = k(K_{\text{ATRP}})\left(\frac{[P_nX][\text{Cu}^{\text{I}}]}{[\text{Cu}^{\text{II}}][L]}\right) \]  

(Eq 2)

and results in polymers with tightly controlled architectures inaccessible by diffusion-controlled radical polymerization. This equilibrium is set by many factors, chief of which is the ratio of metal catalysts; throughout the course of the polymerization, the lower oxidation state metal (\(\text{Cu}^1\)) complexes with a ligand (L) and abstracts the halogen atom (X) from the alkyl halide, forming a radical and initiating polymerization. Rapid deactivation of radical via halogen-capping (PnX) prevents the reaction from terminating and simultaneously limits rate of propagation, lowering polydispersity. By changing the ratio of higher and lower oxidation state transition metal catalyst within the polymerization mixture, the rate of this polymerization can be precisely controlled, though of course ligand identity (i.e. bidentate < tridentate), monomer (M) identity (i.e. acrylates < methacrylates), and solvent (i.e. aprotic < protic) also contribute to setting equilibrium (\(K_{\text{ATRP}}\)) and therefore affect rate.\(^{30,31}\) Equilibriums are set such as to strongly favor the deactivated species, limiting inopportune termination and lending ATRP high levels of control over polymer length, polydispersity, topology, composition, and grafting density.\(^{32}\)

ATRP is not the only controlled radical polymerization method; there are several others tailored to various synthetic needs. Nitroxide-mediated polymerization (NMP) relies on the homolysis of the C-O bonds in alkoxyamines to provide a persistent radical and give a highly stable, well-controlled polymerization, and is popular for sterically hindered monomers such as styrene. RAFT, reversible addition-fragmentation chain transfer, relies on thiocarbonylthio compounds such as thiocarbamates (aka RAFT agents) to form an adduct radical with a
propagating polymer chain, resulting in a fragmentation of the RAFT agent and releasing another radical into the reaction solution to initiate a new polymer chain. Alternately, the RAFT agent has already interacted with a propagating chain, and when confronted with a new propagating chain, the original group is fragmented off. In this way, the radicals are “shared” among the propagating chains, lowering polydispersity. There are several other iterations of living polymerizations, some of which are more robust than others, but all of which extend the lifetime of the propagating chain at least 2-3 orders of magnitude compared to free radical polymerization and all of which do this by achieving near-simultaneous initiation of the majority of chains and spend the majority of time deactivated.\textsuperscript{32,33} Among these, ATRP continues to stand out, owing to its versatility, its broad requirements (no specialty chain transfer agents or mediators necessary), and its general robustness (can be made insensitive to air, can be carried out at room temperature, at ambient pressures, in most solvents). Therefore, ATRP was selected as the focus for the surface-tethered polymer brush modification route.

Figure 2.1: Example of an atom-transfer radical polymerization in bulk

Using Cu\textsuperscript{I}/Cu\textsuperscript{II} as a catalyst, 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA) as an example ligand, and 11-(2-bromo-2-methyl)propionyloxyundecyltrichlorosilane (BPOTS) as an example initiator, a simplified version of the atom-transfer radical polymerization
mechanism is displayed in Figure 2.1. Once the radical is formed by the abstraction of the capping halogen atom (bromine in this case), the radical can either be deactivated by the same halogen, or react with the vinyl monomer in solution and propagate the polymerization. Equilibrium strongly favors the deactivated species.

2.2. Atom-Transfer Radical Polymerization

Pioneered independently in 1995 by both Matyjaszewski and Higashimuras groups, the development of controlled radical polymerization to access low polydispersity, high molecular weight polymers has been employed to meet several highly specific polymer systems or applications, including thermosensitive valves, ultrahigh molecular weight poly(styrene), and block co-polymers for antimicrobial hull coatings. Indeed, the expanding of ATRP procedures in the last decade have given researchers access to several classes of homo-polymers and co-polymers, and have been adapted for industry use with “green” and aerobic iterations of ATRP, including ARGET (Activators ReGenerated by Electron Transfer) and ICAR (Initiators for Continuous Activator Regeneration). These variations require lower concentrations of catalyst and include consumable reductants or radical generators in order to make the reactions easier to perform or more environmentally friendly. Though ATRP can be conducted with most transition metals, the Cu(I)/Cu(II) system is well-documented in literature and has easily controlled redox chemistry, which makes it ideal for research. While not perfect on an industrial scale, which balks at the use of such high concentrations of metal (normally in ratios of 500:1 monomer:metal), Cu(I)/Cu(II) is a robust model system for research and was used exclusively in these experiments.
The highly controlled nature of ATRP makes it ideal for specialty applications, particularly for precisely controlled, thin surfaces. Microfluidics is one developing field which has benefitted greatly from SI-ATRP, making ultrathin hydrophobic or antifouling coatings. Selectively reactive surfaces, such as the ones used in the highly sensitive detection of pesticides, are another application which is benefitted by the use of ATRP – as nanosensors grow in popularity, there is a corresponding need for tight chemical gradients and high spatial control to direct and separate analytes to the sensor surface. ATRP is an excellent way to modify the highly confined architectures of nanopores without obstructing flow, and lends the pores increased chemical stability and providing interesting adsorption and catalytic opportunities. In nanoparticle synthesis, low molecular weight distributions are important for retaining and investigating exact optical and mechanical properties; therefore, when looking to tune nanoparticle properties such as wettability or charge, access to highly controlled organic modifications such as SI-ATRP is critical for keeping the nanoparticle distribution homogenous. This is also a facile route to complex nanoparticle composites or architectures otherwise difficult to achieve with free radical polymerization and inorganic synthesis. ATRP is a straightforward way to produce complex mixtures of copolymers on a single surface (a near-impossible techniques with free radical polymerization), and is amenable to many surface materials, including cellulose, aluminum, germanium, silicon dioxide, and gold, making SI-ATRP broadly applicable to fields ranging from biosensors (i.e. immobilizing select biomolecules to maximize their individual functionalities) and mechanical engineering (i.e. altering the tribological properties for lubrication).

The versatility of ATRP has been excellent in that it adaptable to many needs, but is also challenging in that an equivalently flexible general ATRP synthesis has not been presented.
As discussed previously, each monomer has its own activity, and the rate of polymerization is highly dependent on ratio of catalysts. As each researcher is interested in optimally solving one specific problem, concentrations of monomer, metals, ligand, and solvent will vary tremendously across different studies according to individual interest, making it difficult to effectively translate one ATRP synthesis to another. Reagents and conditions which worked well for a hydrophilic polymer such as p(DMAEMA) might work poorly for a hydrophobic polymer such as p(St), but some conditions for hydrophilic p(HEMA) may work better for p(St) than p(DMAEMA). Growth profiles will also vary, with low activity monomers suffering from slow initiation and exponential growth while high activity monomers behave linearly. These mental exercises in conversion between syntheses diminish the advantages of ATRP, otherwise considered one of the greatest and most accessible advances in polymer chemistry. This project seeks to address that disparity by presenting a unified polymerization approach on seven monomers of interest, two of which have no established synthetic procedure currently in the literature.

Here a generalized ATRP approach is presented, which is effective for polymer growth both in bulk and off surfaces, for both hydrophilic and hydrophobic polymers. While the polymers grown might not be grown under optimal conditions (and for specific applications, a reader may find better resources in a more tightly focused paper) the polymers are grown effectively and in a reasonable amount of time (6 hrs). The procedure provided within allows ATRP to be exactly what it was intended for: a straightforward way for everyone to grow controlled polymers on a reasonable timescale.
2.3 Experimental Outline

Materials: 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA), copper (II) bromide, copper (I) bromide, 2-(4-Morpholino)ethyl 2-bromoisobutyrate (ME-Br), 2-(Dimethylamino)ethyl methacrylate (DMAEMA), methyl methacrylate (MMA), 2-Hydroxethyl methacrylate (HEMA), styrene (St), and propargyl acrylate (PPA) were purchased from Sigma Aldrich. Monomers were purified of polymerization inhibitors by filtration through the appropriate inhibitor removal column. N-Isopropylacrylamide (NIPAM) was purchased from TCI America and dissolved in methanol (99.8% from VWR International), then passed through a de-inhibition column. Neutral aluminum oxide was purchased from Alfa Aesar. 11-(2-bromo-2-methyl)propionyloxyundecyltrichlorosilane (BPOTS) was purchased from Gelest, Inc.

Bulk Polymerization: All glassware was stored in an oven to ensure dryness. ME-Br was used for the initiator in bulk reactions as it makes for convenient NMR analysis. Under argon, a round bottom flask was charged with Me-Br and left on a Schlenk line, then left under vacuum. Monomer in solvent was measured out and added to a two-neck round bottom flask equipped with a stir-bar, then opened to the Schlenk line under argon flow. The solution was stirred slowly and degassed with argon for ten minutes using a large pore glass frit. After degassing, copper (II) bromide, HMTETA, and copper (I) bromide were added, and the solution was sealed and stirred for ten minutes. 40 mL of the monomer-solvent solution was transferred to the initiator-charged reaction flask. The reaction ran continuously for 6 hours with a 3 mL aliquot taken every 30 minutes at room temperature for NIPAM, DMAEMA, MMA, HEMA, and PPA, and at 100° C for styrene. The aliquots were immediately purified by passing through neutral alumina columns to remove the copper catalyst. The samples were then dried thoroughly under vacuum.
Self-Assembled Monolayer Formation: Silicon wafers were activated using piranha (Caution! Piranha solution can react strongly with organic compounds. It should be handled with extreme caution). The activated silicon wafers were completely submerged in a bulk solution of 1 mM BPOTS in hexane. The wafers were then sealed off from oxygen and stored in a desiccator for 24 hours, allowing excess time for surface monolayer formation. After this, the wafers and chips were sonicated hexane and dried under nitrogen. Once dry, spectroscopic ellipsometry was used to assess monolayer quality, with the BPOTS monolayer determined to be 2.1 nm.

Surface-Initiated Polymerization. BPOTS-modified wafers are then sealed in a round bottom flask and left on a Schlenk line under vacuum. Monomer in solvent was measured out and added to a two-neck round bottom flask equipped with a stir-bar, then opened to the Schlenk line under argon flow. The solution was stirred slowly and degassed with argon for ten minutes using a large pore glass frit. After degassing, copper (II) bromide, HMTETA, and copper (I) bromide were added, and the solution was sealed and stirred for ten minutes. After polymerization, wafers are immediately removed from the monomer solution and sonicated in THF, IPA, and water, then dried under nitrogen flow. A contact angle measurement was made on each wafer once again and the wafers are stored in ambient conditions until further characterization.

Ellipsometry Measurements. The thickness of each dry initiator layer and polymer brush on the silicon substrates was determined using a J.A. Woollam M-2000 spectroscopic ellipsometer at room temperature (Lurie Nanofabrication Facility, University of Michigan, Ann Arbor, MI). Data was collected between the wavelengths of 190-1700 nm at three angles of incidence (65°, 70°, 75°) and the 300-900 nm region was fit to a Cauchy model. The SiO₂ layer was set at 20 Å, and all parameters were allowed to vary. n and k were compared to literature values in the visible
region to assess goodness of fit. 3-5 measurements were taken per sample to determine polydispersity of the surfaces.

Gel Permeation Chromatography: The molecular weight and polydispersity of each sample in the bulk polymerization was determined by GPC. A Waters system, equipped with a 1515 isocratic pump, a 2414 refractive index detector, and 2998 photodiode array detector, in addition to a miniDAWN TREOS 3-angle laser light scattering detector (MALLS, Wyatt Technology, CA). Separations were performed at 23° C using a mobile phase of DMF containing 0.1 M LiBr. The MALLS detector was calibrated using pure toluene and used for the determination of the absolute molecular weights, with the detection wavelength of TREOS set at 658 nm. The molecular weight of all polymers was determined based on the dn/dc value of each sample calculated offline by using an internal calibration system processed by the ASTRA 6 software (version 6.1.1, Wyatt technology, CA). The obtained data points were imported into Excel 2016, plotted, and saved as vector image files (*.ai) for coloring and annotation in Adobe Illustrator CS6.

Contact Angle Measurements: Measurements for dry wafers were taken both before and after the polymerization using a Rame-Hart Instrument Co. goniometer model 120-F0 at room temperature and analyzed with DROPimage CA software. 15 µL of 18.6 MΩ water was deposited directly onto the wafer surface with the flat contact angle being recorded.

Nuclear Magnetic Resonance: NMR spectra were recorded at room temperature on a Carver B500 NMR Spectrometer, operating at 500 MHz and 125 MHz for ¹H and ¹³C acquisitions, respectively. NMR spectra were processed using MestReNova software and chemical shifts are reported in ppm and referenced to the corresponding residual nuclei in the deuterated solvent.
Control samples were prepared using the 2-(4-Morpholino)ethyl 2-bromoisobutyrate (ME-Br) initiator, which was selected because the morpholine group provided a convenient NMR label. Comparison of the peak integrals due to the methacrylate backbone with those of the terminal morpholine group (unique signals at 2.3 and 2.6 ppm) allows the $M_n$ to be calculated for each sample.

X-ray Photoelectron Spectroscopy. The thickness of representative dry initiator layer and polymer brushes on the silicon wafers was also determined using a Kratos Axis Ultra with an aluminum source.

X-ray Reflectometry. The thickness of representative dry initiator layers and polymer brushes on silicon wafers was determined finally using a Panalytical/Philips X’PERT MRD system.

2.4 Results and Discussion

All seven polymer brushes were grown under nearly identical conditions in bulk and surface-tethered; polystyrene had to be grown under elevated temperatures to overcome the inherently low monomer activity. In regards to the surface-initiated brushes, in addition to length, the on-surface polydispersity was of great interest; typically, grafting-from approaches allow for incredibly dense and highly mechanically stable polymer surfaces when compared grafting-to approaches (in which the brushes are pre-formed using bulk ATRP and then modified to have a surface-reacting end group), but what was found was that this was tremendously dependent on the initiator monolayer. When the monolayer was deposited with either too high of a concentration (greater than 1 M) or over too short a period of time (less than 24 hours), the monolayers were patchy, and the resulting polymer brushes varied greatly in length. For the bulk polymerizations, while it would have been ideal to use BPOTS as the initiator (so that initiator
activity would be kept constant through all reactions), free silane is water-sensitive, and so Me-Br was selected instead.

Spectroscopic ellipsometry was used to probe brush length over time and gel permeation chromatography was used to investigate bulk polymer growth over time. Although MALDI is also popular amongst polymer scientists, GPC’s widespread adoption and better polydispersity characterization make it a superior technique for the purposes of this study. It is possible to correlate the molecular weight results to chain length (keeping in mind the different initiator activities) by using MarvinSketch or ChemDraw to estimate the length of a monomeric unit and then back calculate the molecular mass of the chain.

An example of one of each of the analysis methods is given below (except XPS, which is in progress with KAS).
A). An example of the raw data and the fitting process for spectroscopic ellipsometry. Data is collected across a 400 nm window at three angles of light and then fit. The data is only as accurate as the model when it comes to spectroscopic ellipsometry, and here we rely on a non-absorbing Cauchy model, which is ideal for thin organic films. The model tracks nicely with the data, indicating a good model selection.
Figure 2.2 (cont.)

B). Thickness of a p(DMAEMA) brush with time. The error bars are from three points taken on the same wafer; per wafer, 3-5 data points are collected to investigate sample polydispersity.

Figure 2.3: Gel permeation chromatography example – bulk p(MMA) and p(DMAEMA)

A). Refractive index profile from gel permeation chromatography; the absorption peak shifts as samples grown for different amounts of time (1 hr, 2 hr, 3 hr) pass through the column.
Figure 2.3 (cont.)

B). The change of molecular weight with growth time in an ATRP polymerization is shown using the axis on the left, and the change in polydispersity index is shown on the right. This particular example came from a loosely controlled p(DMAEMA) attempt in which higher catalyst concentration resulted in increasing polydispersity beyond tolerated levels for an ATRP.

One of the advantages of using ATRP is that growth proceeds linearly with time; non-linear growth can be attributed to either slow initiation, as is common with less active monomers (styrene, the acrylates), or to catalyst poisoning (i.e. by exposure to air). This linear growth is valuable in targeting specific polymer brush lengths; though they grow at different rates according to their individual activities, by determining the growth profile over six hours, lengths can be projected out for later time points, important for applications where thicker brushes are preferred (such as for anti-biofouling surfaces).
Reaction progress can also be monitored by nuclear magnetic resonance; Figure 2.4 shows the Me-Br initiator peaks disappearing from the zero timepoint sample and the subsequent growth of the polymer peaks as the reaction proceeds. Though generally unnecessary, NMR can also be used to identify the polymer and to calculate molecular weight (via end group analysis).
Raman spectroscopy was analogously used on the surface-initiated polymer brushes in Figure 2.5, as each polymer brush as a unique spectroscopic signature. These polymer brushes being quite thin, the signals can be weak, but can still confirm brush identity and could be used in the future to distinguish between different polymers on graded brush surfaces.
Finally, XRR was used to independently confirm brush lengths, as ellipsometry does rely on the user’s knowledge of both the sample and appropriate model parameters and can therefore give user-induced errors in length. It was found that XRR correlated well to ellipsometric fits (in the above example, ellipsometry put sample thickness at $68 \pm 5$ nm and XRR at $67 \pm 3$ nm), indicating that the model parameters used for ellipsometry were reasonable.
2.5 Conclusions

In conclusion, a unified synthetic method for atom transfer radical polymerization, in both bulk and surface-initiated, continues to be investigated. Several common polymers, including p(DMAEMA), p(HEMA), and p(MMA) have been completed and characterized, while less common polymers such as clickable p(azPMA) and p(PA), are still in progress. Using a mixture of water and methanol was found to be the most versatile as both solvents are cheap and solvate a wide range of monomers and corresponding polymer systems (at least for the molecular weight regions being accessed); solvent removal is also straightforward and simple compared to other common ATRP solvents such as DMSO and THF. Copper, being the most common in academic research settings, was selected due to the rich literature on its activity in various ATRP reactions, and HMTETA was selected for its very average level of activity (accessible to both high and low activity monomers). This system is robust towards skill level and background knowledge.
Chapter 3: Polymer Brush-Modified Surfaces for Signal Enhancement

Notes and acknowledgements: This chapter has been reproduced from the original paper “Polymer Brush-Modified Microring Resonators for Partition-Enhanced Small Molecule Chemical Detection” (Stanton, Alexandria L.D., Serrano, K.A., Braun, P.V., Bailey, R.C. ChemistrySelect 2017, 2, 1521-1524). It has been reproduced here with permission from Wiley-VCH © 2017.

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3.1 Motivation

As discussed in Chapter 1, there is a tremendous need for robust, sensitive, and selective bulk property detectors. In particular, when considering molecules for which there is an overlapping need not only for sensitive detection, but also real time detection, such as in the case of chemical warfare agents, there is a great interest in silicon photonic microcavity-based sensors, owing in particular to their high sensitivity and large dynamic range. Furthermore, the intrinsic scalability of silicon microfabrication might allow for widely deployed sensor array networks. However, these devices require modification for enhancing their sensitivity towards small, dilute organic analytes; by occupying the sensing region of the ring resonators with an organic layer, it is possible to increase analyte partitioning into this stationary phase out of an aqueous mobile phase.
3.2 Previous Work and SI-ATRP

Previously, microring resonator arrays were modified using surface-initiated atom-transfer radical polymerization (SI-ATRP) to grow polymer brushes directly from the ring surface, and brush growth could be tracked in real time directly from the resulting shift in resonance wavelength. The brush, zwitterionic p(SBMA), was thoroughly characterized with atomic force microscopy and was shown to have identical thickness and grafting density to a polymer brush grown under equivalent conditions on a flat silicon dioxide surface. This confirmed that no special corrections needed to be made in comparing polymer brush characteristics on a silicon wafer and the microring resonators.

SI-ATRP was an ideal technique to modify the microring resonators as it lends a high degree of structural control, brush composition, and chain length, as is important when working with nano-sized sensors intolerant to high polydispersity. Using ATRP-based organic modifications to change the sensor surface chemistry was therefore one main way to approach enhancing the sensitivity and molecular selectivity through non-covalent molecular interactions. With light being confined within the microring waveguide via total internal reflection and the majority of the active sensing volume within 100 nm of the ring surface, ATRP-grown polymer brushes are particularly attractive as a general approach to organic surface modification, as they can conveniently be grown to thicknesses of ~100 nm with amenability to a diverse set of functional group chemistries. Notably, thicker polymer layers deposited via drop casting or spin coating would be limited by slow response times and relatively poorer sensitivity. The polymer brushes serve to localize molecular species within the evanescent field of the sensors, significantly increasing the sensor response by 1-2 orders of magnitude for given concentrations of analyte and providing a pathway towards greater sensor selectivity.
3.3 Experimental Outline

Using a procedure adapted from Chapter 2, three types of polymer brushes were grown off rings: p(MMA), p(DMAEMA), and p(NIPAM) (Figure 3.1). First, the silicon microring resonator chips were cleaned using oxygen plasma. Then, self-assembled monolayers of the initiator 11-(2-bromo-2-methyl)-propionyl undecyl trichlororosilane (BPOTS) were formed on the substrates by immersion in a 1 mM hexane solution for 24 hours. After being rinsed in fresh hexane and dried under a nitrogen stream, the microchips were placed in a reaction vessel. 1,1,4,7,10,10-hexamethyltriethylene tetramine (HMTETA) was used for the ligand and standard air-free techniques were used to transfer appropriate ratios of [monomer]:[Cu(I)]:[Cu(I)]:[ligand] into the reaction vessel. The polymeric substrates were rinsed with THF, IPA, and H₂O and then dried under a stream of nitrogen. Dry polymer thicknesses were measured off a silicon dioxide wafer derivatized in the same reaction vessel, using single wavelength ellipsometry (Gaertner L116C). The modified chips were exposed to the analytes via integrated microfluidics within the Genalyte Maverick M1 optical scanning instrumentation. Four microring resonators were monitored to determine both either bare- or polymer brush-modified sensor response, while four occluded rings were used for real-time temperature correction. The microring resonators monitored per chip were chosen based on which cluster of rings displayed the lowest standard deviation in response to caffeine standards, per chip. The sensor responses are measured in real-time (see Figure 3.6 for a representative trace) and extracted resonance wavelength shifts averaged over a suitable time period are plotted in Figures 3.2-3.4 for exposure to different small molecule analytes. Please note the ATRP procedures referenced above were adapted from several iterative experiments before the advent of the generalized ATRP approach outlined in Chapter 2, and the thickness analysis not as rigorous (while single wavelength spectroscopy is
frequently relied upon, and was in this case, spectroscopic ellipsometry should always be used when possible).

**Figure 3.1: Sensor functionalization process**

A). Cartoon representation of an analyte being localized within the sensing region, resulting in a shift in local refractive index (black line shifting to red line).

B). Bare chips are activated with oxygen plasma followed by chemical grafting of the initiator monolayer. Surface-bound polymer brushes are then grown from the sensor surface by ATRP. This process allows for the brushes to be covalently bound to the surface in a reproducible and highly controlled manner.

**3.4 Results and Discussion**

This concept of using polymer brushes to enhance sensitivity was first investigated using the common pharmaceutical standards caffeine and acetaminophen. Hydrophilic p(NIPAM) (43 nm dry thickness), and hydrophobic p(MMA) (24 nm dry thickness) polymer brushes were grown off the microring resonator arrays using SI-ATRP and then exposed to water-based solutions of each standard using integrated microfluidics as described previously (Figure 3.2).
Figure 3.2: Enhancement of pharmaceutical standards on ATRP modified ring resonators

A). Resonance wavelength shifts measured for p(NIPAM)- and p(MMA)-modified microring resonators upon exposure to 10 mM aqueous solutions of caffeine and acetaminophen. The responses from bare microrings (20 pm for caffeine and 27 pm for acetaminophen) was subtracted to remove bulk refractive index effects. Error bars represent the standard deviations from four individual microring responses from a single detection experiment.
B). Non-corrected resonance wavelength shifts for caffeine and acetaminophen detection, including bare- and two sets of polymer brush-modified microring sensors exposed to 10 mM aqueous solutions of both analytes.

C). Percent detection enhancement values noted on plot were determined by dividing polymer brush-modified responses by bare microring sensor response.

Initial observations reveal enhanced response of the analytes on the modified rings compared to bare, unmodified rings, due to localization of the organic molecules within the organic brush on microring surface. In order to just focus on the amount of analyte partitioned into the polymer brush, and not bulk refractive index changes in solution, the response from unmodified sensors was subtracted from the polymer brush-modified microrings, as shown in Figure 3.2 A. (Non-subtracted resonance shift data, as well as percentage enhancement compare to unmodified sensors, are displayed in Figure 3.2 B and C). Analyte enhancement is observed within both polymer brushes; however, acetaminophen shows a significantly greater response when interacting with the p(NIPAM) brush, with a 10-fold larger resonance shift compared to the response of p(MMA)-modified microrings, and 400% enhancement over un-modified sensors.

The enhancement is almost certainly due to partitioning of the small molecule analyte into the organic layer. While there are many factors which can drive partitioning, the effect of solvent and brush swelling is likely important. p(MMA) is hydrophobic, and swells only 2% in water, in contrast to the much more hydrophilic p(NIPAM) brush, which likely extends further into solution, providing a more accessible construct for chemically-selective analyte partitioning.
Further exploring the role of brush extension and response, the partitioning of Bisphenol A (BPA), a toxic industrial chemical, into p(NIPAM) (230 nm thick) and p(MMA) (250 nm thick) polymer brushes was probed in both aqueous and 90:10 water:acetonitrile solutions.
Figure 3.3: Effect of solvent on enhancement of bisphenol A on modified ring resonators

A). Resonance wavelength shifts measured for p(NIPAM)- and p(MMA)-modified microring resonators upon exposure to 10 mM solutions of Bisphenol A prepared in both water and a 90:10 water:acetonitrile mixture. The responses from bare microrings (25 pm and 152 pm for water and water:acetonitrile, respectively) was subtracted to remove bulk refractive index effects. Error bars represent the standard deviations from four individual microring responses from a single detection experiment.
Figure 3.3 (cont.)

B). Non-corrected resonance wavelength shifts for glyphosate detection, including bare- and two sets of polymer brush-modified microring sensors exposed to 10 mM solutions of Bisphenol A.

C). Percent detection enhancement values noted on plot were determined by dividing polymer brush-modified responses by bare microring sensor response.

For both brushes, the response to a 10 mM solution of BPA was increased in the acetonitrile-containing solvent. Again, the more hydrophilic p(NIPAM) brush showed a larger response, but the addition of a small amount of organic solvent, which presumably swelled both polymer brushes, led to a substantial increase in observed resonance wavelength shift for both brushes. Interestingly, the relative percent enhancement between p(NIPAM) and p(MMA) remained constant (~9-fold larger for p(NIPAM)) in both solvent systems (see Figure 3.3).

These initial experiments indicate the possibility of using polymer brush-modified microring resonators for small molecule, organic compound detection, and the potential to tune analyte sensitivity and selectivity by altering brush:analyte:solvent interactions. As a preliminary test of the applicability of polymer brush-modified microring resonators, the detection of 4-methylumbelliferyl phosphate, a CWA simulant was investigated. Three different types of polymer brushes were grown on microring resonator array substrates: p(NIPAM) (43 nm thick), p(MMA) (24 nm thick), and p(DMAEMA) (26 nm thick). First, four different concentrations of 4-methylumbelliferyl phosphate were separately flowed across the differentially-modified sensors, with the resonance wavelength shifts (with bare microring response subtracted) shown in Figure 3.4 A-C. Then, the herbicide glyphosate was tested, and it was found that enhanced responses are also observed for this organophosphate (Figure 3.4 D-F).
Figure 3.4: Enhancement of CWA mimics on modified microring resonators
Figure 3.4 (cont.)

A). Resonance wavelength shifts measured for p(NIPAM)-, p(MMA)-, and p(DMAEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of 4-methylumbelliferyl phosphate analytes. Responses from bare microrings were subtracted to remove bulk refractive index effects, and error bars represent the standard deviations from four individual microring responses from a single detection experiment.

B). Non-corrected resonance wavelength shifts for 4-methylumbelliferyl phosphate detection, including bare - three sets of polymer brush-modified microring sensors at four different concentrations of 4-methylumbelliferyl phosphate.

C). Percent detection enhancement values noted on plot were determined by dividing polymer brush-modified responses by bare microring sensor response.

D). Resonance wavelength shifts measured for p(NIPAM)-, p(MMA)-, and p(DMAEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of glyphosate analytes. Responses from bare microrings were subtracted to remove bulk refractive index effects, and error bars represent the standard deviations from four individual microring responses from a single detection experiment.

E). Non-corrected resonance wavelength shifts for glyphosate detection, including bare - three sets of polymer brush-modified microring sensors at four different glyphosate concentrations.

F). Percent detection enhancement values noted on plot were determined by dividing polymer brush-modified responses by bare microring sensor response.
In all cases, a concentration-dependent response is observed, with the p(DMAEMA) brush showing the largest degree of enhancement—at least 20-fold for greater signals compared to other brush chemistries, and 5000+% response enhancement compared to non-functionalized sensors (see Figure 3.4C). Notably, the overall resonance wavelength shifts are much smaller for this analyte, as the refractive index of glyphosate is lower than the aromatic 4-methylumbelliferyl phosphate analyte; however, the effects of bulk refractive index change have been corrected by again subtracting the bare resonator signal. This reinforces the observation that molecular partitioning plays a substantial role in dictating sensor response as higher refractive index analytes partitioned within polymer brush-modified microrings show enhanced sensor response.

Importantly, though, the differential signal measured by the different brush-modified microrings suggests the potential for array-based target identification. Specifically, arrays of differentially-functionalized microrings could potentially, in a single detection experiment, provide both quantitative concentration determination, as well as a target-specific signature that would facilitate agent identification. This could be analogous to the highly successful optoelectronic “nose” arrays, which respond to the subtly different chemical reactivities of volatile organic compounds. The origin of specific intermolecular forces that led to this differential response are beyond the scope of these experiments; however, it could be speculated that the combination of brush and analyte solubilities in the solvent system play an important role in sensor response that could be optimized for particular target agents of interest.

To further investigate the interactions of solution-phase analytes with different polymer brush chemistries, we studied the partitioning of aqueous solutions of methanol, ethanol, and octanol with microring sensors presenting hydrophobic p(MMA) and hydrophilic p(DMAEMA)
polymer brushes; dry thicknesses of 65 and 40 nm, respectively. This set of experiments focused on a single class of small molecule targets—alcohols—and was designed to examine the role of hydrophobicity and polymer solubility in a systematic way. Response of polymer brush-modified and bare microring resonators to aqueous solutions of these alcohols in decreasing concentrations as noted is included in Figure 3.5; pure water and alcohol-containing aqueous solutions are cycled at 10 minute intervals.

Figure 3.5: Alcohol partitioning into polymer brushes

A: Methanol
B: Ethanol
C: Octanol
Figure 3.5 (cont).

A). Exposure to methanolic solutions show large magnitude shifts for p(DMAEMA)-modified rings due to strong solubility of methanol in the polymer brush. The response of p(MMA)-modified rings is equivalent to bare microring indicating no partitioning.

B). Exposure to ethanolic solutions shows similar behavior; strong interactions with p(DMAEMA) and nothing for p(MMA).

C). Exposure to octanolic solutions elicits responses from both p(MMA) and p(DMAEMA)-modified microrings on account of octanol being an interacting solvent for both polymer brushes. Both responses are distinctly different from that of bare microrings.

For methanol and ethanol, both of which are highly water-miscible, the hydrophilic p(DMAEMA) showed large negative resonance shifts, whereas hydrophobic p(MMA) showed a response similar to the blank microring, indicating no analyte partitioning. For octanol, which is significantly more hydrophobic (much less miscible with water), highly differential responses were observed, with p(MMA)-modified sensors showing a positive shift in resonance wavelength larger than the blank ring, while p(DMAEMA) brushes showed a negative shift. The opposite signs of these shifts suggest that the resonance shifts are reflective of partitioning according to intermolecular forces. In this case, this is a combination of solubility and hydrophobicity differences between the analytes and two different polymer brush chemistries.

To help explain these responses it is important to consider the solubility parameters, $\delta$, of the compounds involved in this interaction, which are listed in the table below.$^{49,50}$ Equivalent solubility parameters suggest that compounds are miscible, or are a good solvent combination. First considering the responses of p(MMA), we found that there was no difference in response
from p(MMA)-modified microrings compared with bare microring sensors, and this is consistent with the fact that methanol and ethanol do not interact with p(MMA). However, when exposed to octanol, which has a solubility parameter similar to p(MMA), we see a positive resonance wavelength shift, consistent with the notion that octanol can partition into the polymer brush.

The interactions of the alcohols with p(DMAEMA) is somewhat more complex, and the solubility parameter for this polymer is unknown. However, the hydrophilic nature of p(DMAEMA) and literature reports suggest that both methanol\textsuperscript{51} and ethanol\textsuperscript{52} are good solvents for this polymer. By contrast, one would not expect octanol to be as good of a solvent considering it more hydrophobic nature. p(DMAEMA) is also soluble in water and upon flowing water across these initially dry polymer brushes, brush hydration is observed as a positive shift in resonance wavelength. The addition of both ethanol and methanol leads to a large negative shift in the resonance wavelength. The magnitude of the shift is understandable on account of the high solubility of these alcohols in the polymer brush.

The negative direction of the shift for p(DMAEMA) exposed to ethanol and octanol is explained by the fact that the polymer brush is likely swelling as to extend beyond the evanescent field of the sensor, replacing higher refractive index polymer (n ≈ 1.42) with much lower index water (n = 1.33) and methanol (n = 1.329) or ethanol (n = 1.36). The original p(DMAEMA) brush was 40 nm thick when fully dried, and is expected to be reasonably thicker when hydrated, perhaps even up to ~60 nm. This is already nearly equivalent to the 1/e decay length of the microrings evanescent field sensitivity profile. While the resonators are still

\[
\begin{array}{|c|c|}
\hline
\text{Compound} & \delta ([MPa]^{0.5}) \\
\hline
\text{Water} & 48.0 \\
\text{Methanol} & 29.7 \\
\text{Ethanol} & 26.1 \\
\text{Octanol} & 21.0 \\
\text{PMMA} & 20.0 \\
\text{PDMAEMA} & \text{unknown} \\
\hline
\end{array}
\]
sensitive to refractive index at and beyond this distance from the surface, the relative sensitivity to changes in this region are less than the same RI changes nearer the surface. Moreover, it was previously determined that “ethanol is a more effective solvent for p(DMAEMA) than water.” Therefore additional partitioning of ethanol into the polymer brush would likely lead to additional polymer swelling. Moreover, as mentioned above, as the polymer brush swells beyond into this less sensitive distance from the surface, the extended p(DMAEMA) is replaced by lower refractive index water and alcohol, effectively lowering the n_{eff} sampled by the optical mode and leading to a negative resonance wavelength shift. When exposed to octanol, negative resonance shifts are again observed for p(DMAEMA); however, their magnitude is reduced because octanol is a poorer solvent for this polymer.

It is worthwhile to point out that the responses from p(DMAEMA) upon cycling from water to methanol and ethanol appear somewhat irregular, but the negative shift in the alcohol solution followed by positive shift in water is consistent. The irregularity of the “shape” of the response is something that will require additional studies to fully understand; however, it is perhaps not surprising given the complexities of these solubility/hydration interactions. Also, it is important to note the difference between simple swelling and brush strand dissolution. Many compounds will penetrate a chemical film, simply diffusing in at a rate dictated by penetrant size and brush matrix, but the localized relaxation of the brush in the presence of a penetrant is classified as dissolution. Dissolution of the brush structure is likely concentration-dependent and defined by non-Fickian transport. Our measurement is likely sensitive to brush extension and dissolution as that changes the relative occupancy of the evanescent field by higher RI polymer and lower RI water/alcohols, and the partition kinetics are complex and warrant future studies.
By comparison, p(MMA), which only shows partitioning of octanol, is a glassy polymer, in contrast to p(DMAEMA). Dissolution is more likely to occur in a “Case II” manner where a sharp front distinguishes swollen and unswollen regions, while a front of solvent penetrates at a constant rate. This more well-defined and more limited partitioning may explain the more well-behaved shifts in resonance wavelength. Also, the refractive index of octanol (n = 1.429) is closer to that of the polymer brush so that any volume replaced by this solvent might still support a positive resonance shift.

Figure 3.6: Real time trace of p(NIPAM) brush with acetaminophen solution

A custom program is used to generate the bar graphs displayed in Figures 3.2-3.4; this program works by selecting 15% (by time) of each step, averaging that signal, and then
subtracting it from the averaged 15% of the previous step (the baseline step). In Figure 3.6, a real-time trace showing the actual experimental data collected off the Maverick instruments is shown, with the plateaued region in between the dotted lines indicates the data which is averaged for the bar graphs.

3.5 Conclusions

In conclusion, polymer-brush modified silicon photonic microring resonators were found to exhibit differential chemical interactions with small molecule analytes, enhancing the sensor response in excess of 1000% for some brush-analyte combination, compared to unmodified sensors. Presumably, this enhancement is due to intermolecular interactions that could be optimized to be highly specific and sensitive for particular classes of target analytes. These results are encouraging as the brushes and small molecules selected represent several different, generally-relevant classes of analytes.
Chapter 4: Real-Time Polymer Brush Dynamics Probed Using Microring Resonators

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4.1 Motivation

The dissolution behavior of polymers and polymer brushes is of critical importance to multiple industries. The dynamics, timescale, and extent to which a polymer dissolves in certain conditions dictate its use in drug delivery, microlithography, and water and chemical resistance. Being of such high industrial importance, the process by which polymers dissolve has been investigated thoroughly using differential scanning calorimetry, FTIR-ATR, atomic force microscopy, ellipsometry, among many other viscoelastic and stress related measurements. These techniques typically probe the optical or mechanical properties of the polymer over time and can suffer from a number of failings, including the need for bulk sample or lack real-time information, which obscures the dissolution dynamics.

4.2 Polymer Dissolution

Bulk polymers go through one of two general processes when dissolving: A), penetration of the free volume of the polymer, gelling of polymer in response to penetrant, and chain
disentanglement leading to full dissolution (external osmotic pressure is relieved by mass transport into the polymer) and B). penetration of the free volume of the polymer followed by crazing (osmotic stress at the interface of the solid and liquid builds too quickly) (See Figure 4.1). The dissolution process will be dictated by polymer properties such as glassiness and tacticity, molecular weight and polydispersity, solubility/solvent properties, polymer hysteresis, the dissolution process (i.e. is the sample agitated? Heated?).

Figure 4.1: Bulk polymer dissolution profile representations

A). Penetration of the free volume of the polymer (pink square), gelling of polymer in response to penetrant (leading to a two, or more, phase polymer, represented in purple, with unpenetrated polymer in pink), and chain disentanglement leading to full dissolution (small pink squares surrounded by penetrant).

B). Penetration of the free volume of the polymer (pink square), followed by crazing and/or cracking (pink square with purple lines), and even losing parts of the polymer to the surrounding solution (without dissolving, pink shapes surrounded by penetrant).
The glass transition point of the polymer (T_g) is a range of temperatures over which the mobility of the polymer chains within the polymer increases considerably (unlike melting, there is no phase change). Correlated to diffusion, it is easy to picture that high T_g materials are less diffusive, and therefore usually more difficult to dissolve as the solvent molecules cannot as free penetrate the polymer structure. Tacticity, which describes the orientation of the monomeric units relative to each other within a polymer, is also a contributing factor in dissolution, with highly ordered isotactic or heterotactic polymers being more difficult to penetrate than atactic.

Besides this, dissolution rate is directly correlated to polymer weight; lower molecular weight polymers tend to have slower dissolution profiles compared to their bulk counterparts, and to complicate matters further, this relation does not trend linearly (increasing polymer molecular weight will not increase the diffusion rate by the same amount). Polydispersity has an impact as well, with highly polydisperse samples (such as might be accessed using free radical polymerization) dissolving much more quickly than monodisperse polymers (such as might be accessed using a controlled radical polymerization). Considering this from a film or surface-tethered perspective, thicker polymers are likely to dissolve faster than thin polymer of the same material purely because of the increase in number of access sites (more likely to have pores, pre-existing cracks, etc.).

The chemical properties of the polymer relative to the solvent also contribute, with "like dissolving like" holding true just as it does for non-polymers. This is a deep subset entirely on its own, but on the highest level it is important to consider that the relative contributions of van der Waals forces, dipole interactions, and hydrogen bonding from both the solvent and the polymer affect the solubility of the polymer, and that the more closely the sum of these contributions (the Hansen solubility parameters δ) are matched, the better the solubility. There are many variations
on this and dozens of exceptions, but essentially if the polymer and the solvent have small differences between their solubility parameters, this is a good indication that the polymer is soluble in the solvent.\textsuperscript{73,74}

The polymer’s dissolution rate and profile are also affected by the polymer’s history. Thermal annealing, previous solvent exposure, and casting techniques all affect the physical properties of the polymer, including how quickly and which solvents diffuse through the polymer.\textsuperscript{75} Obviously, the conditions under which a polymer is dissolved also matters: dissolution can be increased by increasing solvent temperature, agitating the solution, or even radiation exposure. Agitation (such as stirring) in particular is interesting to consider as the process by which it increases dissolution is by decreasing the surface layer thickness and therefore increasing external osmotic pressure. This is important to consider when setting up a dissolution experiment: is the experiment under static exposure, or flow?

None of these contributions is truly independent of each other, and the dissolution process is greatly affected by other parameters such as penetrant size and polymer morphology. An important take away point is that bulk polymer could be expected to have tremendously different dissolution profiles compared to polymer brushes, especially polymer brushes produced via surface-initiated atom-transfer radical polymerization. A controlled radical polymerization such as ATRP leads to polymers which presumably have low entanglement, low polydispersity, low molecular weight, and are surface-bound, affecting the dissolution the equilibrium of the dissolved polymer free in solution vs. undissolved polymer still gelled or unpenetrated. A considerable contribution to diffusion models is the assumption of flux or mass transport of the polymer and its distribution into the solvent, something which does not occur in surface-tethered
films. As such, the dissolution mechanics could be expected to vary widely from the corresponding bulk polymers.

The ability to probe minute physical changes in real time, in tandem with the ability to form thin, dense, and well-controlled polymer brushes directly within the sensing region makes this platform a welcome addition to the bevvy of techniques used to study this fascinating problem of polymer dissolution. Here the dissolution process of four polymer systems, poly(methyl methacrylate), poly(hydroxyethyl methacrylate), poly(hydroxyethyl acrylate), and poly(dimethylaminoethyl methacrylate) is investigated by exposure to mixed solvent systems (THF/water) and plasticizer-doped solvents (nitrobenzene, MIBK, DEP). Plasticizers are typically good penetrants which can be added to solvents to improve dissolution rate; they decrease the interactions of the polymer with itself and therefore lower $T_g$ and boost dissolution (see Figure 4.2). The plasticizers selected would therefore be expected to strongly interact with the polymer brushes and increase the dissolution of the brush within the solvent.

Figure 4.2: Relaxing of a polymer brush when exposed to a plasticizer

4.3 Experimental Details

Using a procedure adapted from Chapter 2, four types of polymer brushes were grown off rings: p(MMA), p(DMAEMA), and p(HEMA), and p(HEA). As previously described, the silicon microring resonator chips were cleaned using oxygen plasma, then self-assembled monolayers of
the initiator 11-(2-bromo-2-methyl)-propionyl undecyl tricholorosilane (BPOTS) were formed on the substrates by immersion in a 1 mM hexane solution for 24 hours. After being sonicated in fresh hexane and dried under a nitrogen stream, the microchips were placed in a reaction vessel. 1,1,4,7,10,10-hexamethyltriethylene tetramine (HMTETA) was used for the ligand and standard schlenk techniques were used to transfer appropriate ratios of [monomer]:[Cu(I)]:[Cu(I)]:[ligand] into the reaction vessel. The polymeric substrates were rinsed with THF, IPA, and H2O and then dried under a stream of nitrogen. Dry polymer thicknesses were measured off a silicon dioxide wafer derivatized in the same reaction vessel, using a J.A. Woollam M-2000 spectroscopic ellipsometer at room temperature (Lurie Nanofabrication Facility, University of Michigan, Ann Arbor, MI). The modified chips were exposed to the analytes via integrated microfluidics within the Genalyte Maverick M1 optical scanning instrumentation. Four microring resonators were monitored to determine both either bare- or polymer brush-modified sensor response, while four occluded rings were used for real-time temperature correction. The microring resonators monitored per chip were chosen based on which cluster of rings displayed the lowest standard deviation in response to methyl isobutyl ketone standards, per chip. The sensor responses are measured in real-time (see Figure 4.8 for a representative trace) and extracted resonance wavelength shifts averaged over a suitable time period are plotted in Figures 4.3-4.6.

4.4 Results and Discussion

Methyl isobutyl ketone (MIBK), an industry-favored solvent and plasticizer, is widely used as a plasticizing agent as it is acid-resistant, low viscosity, and has low water solubility. Four increasing concentrations were exposed to the polymer brushes, with water rinses in between each exposure (Figure 4.3).
Figure 4.3: Exposure of methyl isobutyl ketone to four polymer brush types

A). Resonance wavelength shifts measured for p(MMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of MIBK. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.
B). Resonance wavelength shifts measured for p(DMAEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of MIBK. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

C). Resonance wavelength shifts measured for p(HEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of MIBK. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

D). Resonance wavelength shifts measured for p(HEA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of MIBK. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

Though there is a trend with higher molecular weight polymers experiencing more rapid diffusion, MIBK and p(MMA) are known to have unique profiles in which MIBK is an excellent solvent for low molecular weight p(MMA) and worse for higher molecular weights. At low MW, MIBK induces crazing and cracking prior to dissolution, indicating a rapid rate of diffusion through the polymer; at high molecular weights (above 100 kDa), the dissolution occurs more smoothly, first undergoing a gel equilibrium before dissolving completely. Thin brushes of p(MMA) responded much more strongly than thick brushes, correlating well with this behavior.

The comparison of p(HEA) and p(HEMA) is also interesting to consider, with the brushes having very similar properties except for $T_g$ (the acrylate, p(HEA), has a $T_g$ of -15° C,
and the methacrylate p(HEMA) has a \( T_g \) around 57\(^\circ\) C). Low \( T_g \) p(HEA) experienced relatively linear responses to the addition of MIBK, with thicker brushes responding more than thinner brushes, but higher \( T_g \) p(HEMA) had attenuated signal when compared to unmodified rings. p(DMAEMA) \( (T_g \ 19^\circ\) C), experienced strong and negatively shifted response to MIBK when compared to unmodified rings, with the thicker brushes responding considerably more than the thinner brushes. It is important to keep in mind that the direction of the signal (positive or negative) does not particularly matter as it simply indicates whether the local refractive index is increasing or decreasing; with p(DMAEMA) responding so strongly negatively, this could imply that the MIBK is increasing the diffusion of low-RI water into the sensing the region, swelling the brush and lowering the effective refractive index.

Diethyl phthalate (DEP) was popular in the cosmetics and fragrance industries until recently, when the potential neurotoxicity and reproductive toxicity was brought to the forefront. Nevertheless, it is an excellent plasticizer and still widely used as a solvent. Five increasing concentrations were exposed to the polymer brushes, with water rinses in between each exposure (Figure 4.4).
Figure 4.4: Exposure of diethyl phthalate to four polymer brush types

A). Resonance wavelength shifts measured for p(MMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of DEP. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.
B). Resonance wavelength shifts measured for p(DMAEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of DEP. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

C). Resonance wavelength shifts measured for p(HEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of DEP. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

D). Resonance wavelength shifts measured for p(HEA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of DEP. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

Both p(HEMA) and p(MMA) are known to be resistant to dissolving in DEP but have been used to extract it from solutions without undergoing morphological changes. Responses appear to track with length for all brushes, with thinner brushes responding more than thicker brushes, except for p(DMAEMA). Having the most different chemical properties of the four brushes (highly water soluble and amine-containing), the fact that it tracks differently is not surprising.

Next the response of the brushes to a third plasticizer, nitrobenzene (NB), was investigated. As an added plasticizer, nitrobenzene is not as popular as MIBK or DEP, but it is a common internal plasticizer, included as a pendant group for glassy polymers which might
otherwise be too brittle for commercial use. Five increasing concentrations were exposed to the polymer brushes, with water rinses in between each exposure (Figure 4.5).
Figure 4.5: Exposure of nitrobenzene to four polymer brush types

A). Resonance wavelength shifts measured for p(MMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of NB. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

B). Resonance wavelength shifts measured for p(DMAEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of NB. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.
Figure 4.5 (cont.)

C). Resonance wavelength shifts measured for p(HEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of NB. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

D). Resonance wavelength shifts measured for p(HEA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of NB. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

Here is where the divide between p(HEA) and p(HEMA) is most highlighted; p(HEMA) experiences a minor negative response to the presence of nitrobenzene, perhaps indicating that the plasticizer is relaxing the brush and allowing for swelling of the sensing region with water, with the thinner brush responding more strongly than the thicker brush. p(HEA) experiences a small positive response, with a stronger signal from the thicker brush, indicating partitioning of the organic molecule into the brush surface is perhaps more dominant than a change in the brush properties upon exposure to a plasticizer. p(MMA) also experiences this positive response, but is linear neither with thickness nor concentration; there are likely ideal matchings of thickness with NB concentration which maximize signal. p(DMAEMA) continues to exhibit a negative response on roughly the same scale as the DEP experiment.

One thing which stood out after these experiments is that typically the last concentration interrogated had an attenuated response compared to the trend or even compared to the previous
(lower concentration) step. This led to questions on what role hysteresis plays in these experiments and was investigated by repeated exposure to MIBK (Figure 4.6).
Figure 4.6: Hysteresis of the polymer brushes under repeated exposure to methyl isobutyl ketone

A). Magnitude of response from 5 repeated cycles of aqueous 90 mM MIBK, with water rinses in between, measured for p(MMA)-modified microring resonators. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.
B). Magnitude of response from 5 repeated cycles of aqueous 90 mM MIBK, with water rinses in between, measured for p(DMAEMA)-modified microring resonators. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

C). Magnitude of response from 5 repeated cycles of aqueous 90 mM MIBK, with water rinses in between, measured for p(HEMA)-modified microring resonators. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

D). Magnitude of response from 5 repeated cycles of aqueous 90 mM MIBK, with water rinses in between, measured for p(HEA)-modified microring resonators. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

It is clear that the behavior of the polymer in response to the plasticizer changes with exposure; in other words, the thin brushes suffer from hysteresis. The sensitivity to the same concentration of MIBK is diminished as the experiment proceeds, with the rinsing steps failing to reset the brushes to their original structure. This is a subject of particular interest in thin films, which have poorly accessed regions owing to their small pore size and fine structure. As mentioned previously, this slows diffusion compared to the bulk, and it has been hypothesized that hysteresis in thin films is not purely owed to the structural changes from swelling, but also because the micro-sized pores existing in such thin films have restricted access which prevent de-swelling of those regions as the experiment continues. Practically, this strong hysteresis
gave insight into the decreasing sensitivity to increasingly higher concentrations of plasticizers as seen in previous figure. Having observed this, experiments were set up such that all brushes were exposed to all solvents in the exact same order; this does not prevent hysteresis after exposure, but did remove variability in the brush exposure history.

When comparing across brushes, it is interesting to note that there are not distinct trends – longer brushes do not experience significantly more hysteresis than thin brushes, and high $T_g$ brushes do not experience significantly different hysteresis than low $T_g$ brushes, as would be expected. Higher Tg materials typically experience slower diffusion and therefore would have endured less hysteresis over the course of an experiment when compared to a lower Tg material.

Solvents can also be considered to be plasticizing, if thought about as additives in mixed solvent systems. Thus far, all experiments have been conducted in water, which on the bulk is an averagely good solvent for p(DMAEMA) but much less good for p(HEMA) and p(MMA). The presence of non-solvent in a good solvent system can increase the swelling percent up to 3x, but does not increase dissolution. Approaching this from the other side, adding a good solvent to a non-solvent, it could be expected that the brushes would behave differently as they relaxed; solubility rate can change by orders of magnitude with minute changes in solvent composition (even just a few percent!). Using THF as a good solvent, small amounts were added to water and the responses of the brushes were observed (Figure 4.7).
A). Resonance wavelength shifts measured for p(MMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of THF. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.
B). Resonance wavelength shifts measured for p(DMAEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of THF. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

C). Resonance wavelength shifts measured for p(HEMA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of THF. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

D). Resonance wavelength shifts measured for p(HEA)-modified microring resonators upon exposure to various concentrations of aqueous solutions of THF. The signal from the unmodified sensors has been included for reference. Error is taken from the standard deviation of four individual rings.

The response to increasing concentrations of THF was quite dramatic, but there are many factors at work. For one, THF is quite small, and small penetrants as a whole are better at improving dissolution. Interestingly, even though THF should be a better solvent for p(MMA) than MIBK (which frequently induces crazing on a bulk scale), the signal is attenuated compared to the unmodified rings. Again, this could indicate that the p(MMA) is dissolving in the small amount of THF and that water is penetrating the network also, reducing the effective refractive index, but in that case a more dramatic negative signal would have been anticipated at higher concentrations of THF. p(DMAEMA) has limited response compared to the interactions with plasticizers, with the smallest percent inducing the most change. p(HEMA) and p(HEA) respond
similarly to p(MMA) in having slightly attenuated signals when compared to unmodified rings, except for the thickest p(HEMA), with a small negative signal which approaches zero as the concentration of THF is increased. These interactions are quite complex and difficult to fully interpret when examining magnitude only; the shape of the response curves was also investigated (Figure 4.8).
Figure 4.8: Curve fitting for four polymer brushes exposed to tetrahydrofuran

A). Peak shapes for a p(MMA)-modified ring resonator when exposed to four different analytes of increasing concentration (MIBK, DEP, NB, and THF). Error is taken from the standard deviation of four individual rings.
Figure 4.8 (cont.)

B). Peak shapes for a p(DMAEMA)-modified ring resonator when exposed to four different analytes of increasing concentration (MIBK, DEP, NB, and THF). Error is taken from the standard deviation of four individual rings.

C). Peak shapes for a p(HEMA)-modified ring resonator when exposed to four different analytes of increasing concentration (MIBK, DEP, NB, and THF). Error is taken from the standard deviation of four individual rings.

D). Peak shapes for a p(HEA)-modified ring resonator when exposed to four different analytes of increasing concentration (MIBK, DEP, NB, and THF). Error is taken from the standard deviation of four individual rings.

E). A real-time trace of the surface response of a p(MMA)-modified ring resonator to increasing concentrations of THF. The dotted lines indicate the region from which the custom program referenced in Chapter 3 selects data to fit; the curves can be fit between the beginning of a step (first exposure) and the maximum response.

F). Examples of the fitted data (red) overlaying the original curves (black, taken from E). Less and less data is fit as the experiment continues and the signal maximizes faster with higher concentrations.

While the trends in magnitude of response to various plasticizers give some insight into the brush dynamics, there is also a wealth of information within the response shape. As can be seen in Figure 4.8, not all brushes have the same flat plateau signal of a bulk shift (as was observed with the standards caffeine and acetaminophen, for example). Looking at one example
of each brush and taking stock of the response to MIBK, DEP, NB, and THF, some brushes also exhibit a curved response to the analyte, indicating a secondary process besides bulk exposure (i.e. diffusion, relaxation, chain collapse, etc.). By exponentially fitting these curves, the fit can indicate, incredibly roughly, how “strong” the interaction is between the plasticizer and the brush.

4.5 Internal Length Calibration

Having now seen the dramatic effect even small concentrations of plasticizers on polymers, it is important to calibrate the sensitivity of the platform to the polymer brushes. The evanescent field extending off the ring resonators decays exponentially, with 63% of the platform sensitivity being contained within the first 63 nm from the ring surface (Eq 3). Having a polymer brush on the resonator surface localizes analytes within this sensing region, therefore boosting sensitivity of the platform to the small molecule analytes. The enhancement should therefore trend with polymer length; as the polymer occupies more of the sensing region, more of the analyte interacts with the polymer within the sensing region, boosting sensitivity higher and higher.

\[
I(z) = I_0 e^{-2\gamma z}
\]

Intensity of evanescent field \(I(z)\) decays exponentially with perpendicular distance from ring surface \((z)\) with exponential decay constant \(\gamma\).  

(Eq 3)

This enhancement would not be expected to track perfectly; as the polymer extends past the sensing region, enhancements would not be expected to linearly increase, as 1). Sensitivity drops off and 2). Diffusion through the brush and into the sensing region becomes a competing factor. Naturally, polymer brush length is not the only factor on enhancing sensitivity, as RI
differences, polymer glassiness, analyte solubility, and selectivity between the brush and the analyte all contribute, but brush length is a critical factor in maximizing sensor performance.

Water and deuterated water have nearly identical chemical and physical properties (see table to the right); d-H2O has a shorter hydrogen bond length and a slightly lower refractive index in the visible than water, in addition to a slightly higher extinction coefficient.\textsuperscript{78,79} Polymer brushes should respond chemically identically to both solvents, as solubility differences should be negligible. Therefore, if exposed to both solvents, any difference in surface response can be attributed to the refractive index difference between water and d-H2O, providing an internal calibration for brush length and brush sensitivity.

| Table 4.1 – Comparison of H\textsubscript{2}O and D\textsubscript{2}O Constants |
|------------------|---------|---------|
|                  | H\textsubscript{2}O | D\textsubscript{2}O |
| MW               | 18.02   | 20.03   |
| RI (vis.)        | 1.3325  | 1.3282  |
| Absorptivity     | 7.23 x 10\textsuperscript{-9} | 3.05 x 10\textsuperscript{-8} |
Figure 4.9: Exposure of polymer brushes to cycles of water and heavy water

A). Exponential fit of response from four p(MMA) brushes; each brush was exposed to water and then heavy water in repeating cycles up to five times. Error bars are provided in both x (indicating standard deviation in length, as determined using spectroscopic ellipsometry) and y (indicating standard deviation in signal response over five cycles of solvent).
Figure 4.9 (cont.)

B). Reproduced from Reference 20, with permission, copyright Elsevier (2010). “Plot showing the relative resonance wavelength shift for each successive PAH/PSS bilayer as a function of bilayer number. The exponential fit shown in the plot models the decay rate as the polyelectrolyte multilayers grow further from the surface and experience the decreasing evanescent field intensity, as evidenced by the reduced response for each subsequent layer. Error bars represent the standard deviation for n=23 rings.” Previously established exponential decay in signal intensity as the response is gauged at farther and farther distances from the ring surface.

C). Exponential fit of response from three p(HEMA) brushes; each brush was exposed to water and then heavy water in repeating cycles up to five times. Error bars are provided in both x (indicating standard deviation in length, as determined using spectroscopic ellipsometry) and y (indicating standard deviation in signal response over five cycles of solvent).

D). Exponential fit of response from four p(DMAEMA) brushes; each brush was exposed to water and then heavy water in repeating cycles up to five times. Error bars are provided in both x (indicating standard deviation in length, as determined using spectroscopic ellipsometry) and y (indicating standard deviation in signal response over five cycles of solvent).

Four lengths (12 nm, 18 nm, 41 nm, and 114 nm) of p(MMA), three lengths (30 nm, 60 nm, 135 nm) of p(HEMA), and four lengths (10 nm, 22 nm, 44 nm, 211 nm) of p(DMAEMA) were each exposed to five cycles of H$_2$O/D$_2$O. The results, with difference in surface response between H$_2$O and D$_2$O plotted against length, are displayed in Figure 4.9. It can be seen that the signal difference between the two solvents decays exponentially when exposed to hydrophobic p(MMA), but that there is no distinguishable trend for hydrophilic p(HEMA) or p(DMAEMA).
The exponential decay in p(MMA) is precisely as expected, with sensitivity falling off dramatically with small increasing in brush thickness, decreasing the amount of available active sensing region which can be occupied by solvent (comparing Figure 4.9 A and Figure 4.9 B). Considering p(MMA) can swell at most a few percent in water, this decay should depend solely on brush length, and could theoretically be used to determine the brush length of p(MMA) if it was unknown. The signal differences in p(HEMA) and p(DMAEMA), which are hydrophilic and can therefore swell in water, are much more complex as there are several competing factors (i.e. percent swelling vs. length). For cycles of H$_2$O and D$_2$O to lend insight into p(HEMA) or p(DMAEMA) brush length, it would first be necessary to perform liquid cell ellipsometry and monitor changes in brush length as the polymer swelled in both solvents. With a better understanding of the swelling behavior with brush length, perhaps the changes in signal with solvent would resolve into a predictive decay, but otherwise the sensitivity experiments should be limited to glassy, hydrophobic brushes.

4.6 Conclusions

In conclusion, several polymer brushes were exposed to several plasticizing systems and their responses were investigated. It was found the brushes responded differentially to the four plasticizers depending on brush length, plasticizer identity, and solvent system. It was also determined that the polymer brushes experienced hysteresis when exposed to the plasticizer MIBK, which was important to consider when contemplating the applicability of the brushes to field sensing applications. The brush dynamics are complex and still under investigation.
Chapter 5: Surface-Adhered Hydrogels for Enhanced Sensitivity

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5.1 Hydrogels as a Materials Class

Hydrogels are viscoelastic materials which are comprised of a small percent of scaffolding polymer swollen up to 90% in water. Due to their porous nature and swelling capabilities, hydrogels have been used recently for a variety of technological developments. For instance, hydrogels are often used in biomedical engineering as systems of drug delivery due to their ability to control pore radii changes in response to external stimuli. Similarly, controlled volume changes make hydrogels ideal for valves for microfluidic devices. Hydrogels have also been used as separation and purification mechanisms due to their increased adsorptive properties compared to polymers alone. However, one of the main applications of hydrogels is as sensors for a variety of compounds, including relatively benign compounds such as oxygen, glucose, or DNA as well as some more biologically dangerous ones, including organophosphates and neurotoxins. Hydrogels have well-characterized diffusion profiles and are tremendously sensitive to environmental changes such as temperature, pH, or solvent.
5.2 Hydrogels as Sensors

While hydrogels have been used as both biological and environmental sensors, there still are practical limitations to their sensing abilities. Many of the hydrogel sensors currently developed are colorimetric sensors, specifically made of colloid arrays embedded within hydrogels; the volume change of the hydrogel changes the distance between the colloid particles, changing the color of the hydrogel. Hydrogels have also been used as solid-support matrices or concentration matrices for fluorescent probes.\textsuperscript{86,90,91} Though these sensors are quite sensitive and selective past a particular threshold, small concentration changes it can be difficult to differentiate. Interfacing these responsive materials with a universal, robust sensing platform would result in a powerful device widely applicable to many analytical challenges.

Silicon photonic microring resonators are one proposed platform for this problem as they are easy to chemically modify, are bulk property detectors (not reliant on chromophoric or fluorogenic signatures), are highly sensitive to local environmental changes (up to $8 \times 10^{-7}$ RIU), and can be arrayed with individually addressable sensors, making them ideal for graded detection. MRR arrays have been modified with polymer brushes as described previously,\textsuperscript{99} but surface modification of the MRR array using hydrogels is even more straightforward, as instead of relying on an air-sensitive surface-initiated controlled radical polymerization, photo- or thermally-initiated free radical polymerization covalently adheres the hydrogel directly to the surface.\textsuperscript{100} This leaves the microring resonator with a thin hydrogel modification ready for either immediate use or further chemical modification (i.e. to embed the enthalpic gradients for molecular transport\textsuperscript{24}).
5.3 Experimental Outline

Materials: Acrylic acid (monomer), 2,2-diethoxyacetophenone (DEAP), azobisisobutyronitrile, (AIBN), ethylene glycol diacrylate (EGDA), trimethoxysilyl propyl methacrylate (TMPSMA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), glucose, and all buffer materials were purchased from Sigma-Aldrich. N-isopropyl acrylamide and 3A-Amino-3A-deoxy-(2AS,3AS)-β-cyclodextrin hydrate purchased from TCI America. Poly(acrylic acid) at 210 kDa was purchases from Scientific Polymer. All materials were used without further purification. Polymerization inhibitor within the monomers was not removed.

Surface preparation: The microchip is first cleaned with acetone and isopropanol to remove the protective photoresist. After being dried under nitrogen, the chip is activated using oxygen plasma (Harrick Plasma Cleaner PDC-32 G on High, ambient atmosphere) and exposed to 0.1 mM TMPSMA for two hours. The chip is then rinsed in fresh acetone and dried under nitrogen.

Poly(acrylic acid): A monomeric solution containing 20% acrylic acid, 0.6% ethylene glycol dimethacrylate (crosslinker), 5% 210 kDa poly(acrylic acid), 0.01% AIBN (thermal initiator) is poured over the chip such that the active surface is covered. The chips are then put on a hot plate at 80° C for 1.5 minutes. The chips were rinsed in ultrapure water to remove unreacted monomer solution and to swell the resulting hydrogel to completion. The polymerized chips are stored in ultrapure water. Thickness was determined by ellipsometry of dried gels on silicon wafers.

Poly(acrylamide): After cleaning the microchip surface with piranha and functionalizing it with 3-(trimethoxysilyl) propyl methacrylate, an acrylamide monomer/crosslinker solution of 37:1 acrylamide/N,N’-methylenebisacrylamide with a catalytic amount of the photoinitiator 2,2-diethoxyacetophenone was deposited on the microchip surface and exposed to UV-light. The
density of the hydrogel is controlled both by the ratio of acrylamide to bis-acrylamide (the cross linking agent) and by the UV exposure time.\textsuperscript{101}

A note on spin coating: Spin-coating is an excellent surface modification approach and more reproducible in general when compared to our evaporative approach, but was unwieldly when it came to the small chip size (only a few centimeters across), the rough surface (the many surface features on the chip made for an extra challenge) and also the need to obscure the waveguides (if coated with large amounts of polymer, can occlude light from being coupled into the chip). If this synthesis is to be translated to a simpler surface, such as a silicon wafer, it is recommended that spin coating is used preferentially to this evaporative approach. In a related approach, researchers found that spin casting the 210 kDa PAA from DMF resulted in dried gels of about 40 nm.\textsuperscript{102}

\(\beta\)-Cyclodextrin modification: To modify the PAA gel with \(\beta\)-cyclodextrin, an EDC coupling (using a carbodiimide to couple a primary amine) was performed in three different ways. First, an aqueous solution containing 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.78 mmol), 3A-Amino-3A-deoxy-(2AS,3AS)-\(\beta\)-cyclodextrin hydrate (0.06 mmol), sodium chloride (3 mmol), and millipore water (20 mL) was prepared. In one instance, the gelled chip was submerged in this aqueous solution for 12 hours. In the second, a 50 \(\mu\)L aliquot of this solution was spotted onto a portion of the gelled chip and left in a humidity chamber to prevent evaporation for 12 hours. And in the third iteration, the integrated microfluidics of the Maverick instrument system were used to selectively expose only half the gelled ring resonators to the modifying solution. After functionalization, the chips were rinsed with 150 mM sodium chloride solution and pH 7.4 phosphate buffered saline (pH 7.4 PBS, prepared by dissolving phosphate buffered saline tablets in Millipore water).
Phenyl Boronic Acid modification: To modify the PAA gel with PBA, an EDC coupling (using a carbodiimide to couple a primary amine) was performed using the same three methods listed above, except with the modification solution consisting of the following concentrations: 15 mM 1-ethyl3-(3-dimethylaminopropyl)carbodiimide (EDC) and 15 mM 2-aminophenylboronic acid (2-APBA) for 3 hours at pH 4.8 (acetic acid buffer). The chips were washed thoroughly with millipore water.

Microring Resonator data collection: The modified chips were exposed to the analytes via integrated microfluidics within the Genalyte Maverick M1 optical scanning instrumentation. Four microring resonators were monitored to determine both either bare- or polymer-modified sensor response, while four occluded rings were used for real-time temperature correction. The microring resonators monitored per chip were chosen based on which cluster of rings displayed the lowest standard deviation in response to 100 mM NaCl standards, per chip. The sensor responses are measured in real-time and extracted resonance wavelength are shifts averaged.

5.4 Results and Discussion

Modification of microring resonator platform relies on the free radical polymerization of acrylic acid, with high molecular weight, pre-synthesized PAA polymer included as a viscosity agent (See Figure 5.1). After the monomer solution is cast on the surface, the bottom of the surface is rested on a pre-equilibrated hot plate at 80°C. The AIBN included in the monomer solution initiates polymerization, which is allowed to proceed for 90 seconds before being quenched with excess water. The surfaces are dried overnight and analyzed by spectroscopic ellipsometry; polydispersity on the centimeter scale is on the order of tens of nanometers, but on the millimeter scale (corresponding to the chip scale), the height of the gels is ~ 20 ± 5 nm.
Characterization of the thin gels on chip is critical, as thin gels can behave drastically differently than bulk gels. Typically, because there is less ease of access and less strain can be endured, thinner gels swell less than thicker gels, and the extent of this swelling is difficult to determine volumetrically. Using spectroscopic ellipsometry, it was found that the swollen gels were only a few nanometers thicker than the dehydrated gels.

Figure 5.1: Hydrogel adhesion to the microring resonators (MRRs)

Once the gels were adhered to the silicon dioxide surface and the thickness had been characterized, responsiveness of the gel to changes in pH was investigated. Poly(acrylic acid) (PAA) has a pKa ~ 4.2, and the addition of acid is known to cause gel collapse, while base is known to cause gel swelling; when the gel is protonated (under acidic conditions) or the ionized groups are shielded from one another (when exposed to high ionic strength solutions), the gel excludes water and packs more tightly together. When exposed to base, the carboxylic acid groups are deprotonated and the gel increases in water content to shield the charged groups from one another (see Figure 5.2).
A). Real time resonance wavelength shifts measured for TMPSMA (initiator silane)-modified microring resonators upon exposure to pH 8.8 sodium acetate, water, and pH 3.5 acetic acid, with pH 5.3 sodium acetate buffer rinses in between each step.

B). Real time resonance wavelength shifts measured for PAA-modified microring resonators upon exposure to pH 8.8 sodium acetate, water, and pH 3.5 acetic acid, with pH 5.3 sodium acetate buffer rinses in between each step.

The responses were not as distinctive as expected; the silane monolayer had a surprising response to the introduction of base (perhaps removing some non-covalently bound silane from the monolayer assembly?), whereas the polymer responded similarly in terms of magnitude. However, when looking at the acid exposure, TMPSMA has very different responses to water and the acetic acid, as would be expected from their differences in refractive index, but PAA has almost the same response to both. This indicates a physical change in the gel in response to the acid. While others were able to induce tremendous volumetric changes with the addition of acid...
or base, there are also limitations in the robustness of silicon dioxide to hydroxide bases, which limits the compatibility of this platform to these studies (physical changes would undoubtedly be easier to induce with stronger base, for example).

Though the responsiveness of the gels was not quite as expected, the next step, chemically modifying the gel to selectively embed functionality, was attempted. Gel modifications were attempted in three distinct approaches: *in situ*, soaking, and spotting. *In situ* modification consisted of utilizing the integrated microfluidics within the Maverick platform to modify one channel through EDC coupling, while the other channel flowed only water and remained pure PAA gel (See Figure 5.3). Soaking modification involved submerging an entire chip within the EDC modification solution for 12 hours. Spotting modification involved spotting 50 µL of the EDC modification solution on a small portion of the chip (only over 3 clusters of rings, or 12 rings total), leaving the rest of the chip unmodified.

*Figure 5.3: β-Cyclodextrin modification using internal microfluidics*
Figure 5.3 (cont.)

A). EDC-β-CD solution flowed over a TMPSMA surface (initiator silane only, no PAA gel present) and monitored in real time. The final shift difference after modification was 110 pm.

B). EDC-β-CD solution flowed over a PAA gel surface and modified in real time. The final shift difference after modification was 500 pm.

If using the microfluidics internal to the Maverick instrument system, it is possible to monitor surface modification in real time. This is excellent as it allows for saturation to be determined (when modification is complete) and also sheds light on solids deposition; with these salty solutions being exposed to the chip, deposition onto the surface is to be expected, and accounting for this by running the same solution over a silane surface and monitoring change in baseline allows for the true extent of modification vs. deposition to be determined.

Confocal Raman spectroscopy was critical in characterizing the surface modifications – the presence of phenylboronic acid and β-cyclodextrin were otherwise difficult to confirm, and even with Raman, are hard to quantify. One proposed quantification approach was to quantify the thiourea product from the EDC coupling reaction, however the final concentration of that solution was far too dilute for analysis. Raman allowed for distinguishing between modified and unmodified gel regions, and could potentially be used to identify the gradient boundaries when using several of the spotted modifications (See Figure 5.4 for an example of Raman spectroscopy on β-cyclodextrin modified gels).
The thinness and polydispersity of the gels makes Raman difficult, but not impossible, and the modification can be tracked by the slight shifting it the peak centered at 3000 cm$^{-1}$.

With the modification characterized, testing of the differential surface properties between the modified and unmodified gel could begin. β-cyclodextrin is a cyclic oligosaccharide orientated such that they contain a hydrophobic cavity (comprised of glycosidic oxygen groups and carbon backbone) and a hydrophilic exterior (comprised of hydroxyl groups). This allows for reversible interactions with non-polar species, which partition into the cavity and temporarily displace water. In an aqueous environment, this non-covalent interaction has a high dissociation constant; guest molecules are slowed in their transport, as they would be in reversed-phase chromatography, but do not bind permanently to the structure.
Having modified the PAA gel with these β-cyclodextrin pendants, two analytes, fluorescein and nitrobenzene, were selected to probe the response of the modified gels. (See Figure 5.5).

Figure 5.5: Surface response of β-cyclodextrin modified PAA gels
Figure 5.5 (cont.)

A). Real-time resonance wavelength shifts for 0.1 mM fluorescein to an unmodified PAA gel, cycled four times with 100 mM NaCl salt rinse in between. The highlighted region represents the standard deviations from 4 individual microring responses from a single detection experiment.

B). Real-time resonance wavelength shifts for 0.1 mM fluorescein to a β-cyclodextrin modified PAA gel, cycled four times with 100 mM NaCl salt rinse in between. The highlighted region represents the standard deviations from 4 individual microring responses from a single detection experiment.

C). Real-time resonance wavelength shifts for 0.9 mM nitrobenzene to an unmodified PAA gel, cycled three times with water rinses in between. The highlighted region represents the standard deviations from 4 individual microring responses from a single detection experiment.

D). Real-time resonance wavelength shifts for 0.9 mM nitrobenzene to a β-cyclodextrin modified PAA gel, cycled three times with water rinses in between. The highlighted region represents the standard deviations from 4 individual microring responses from a single detection experiment.

Fluorescein is a standard small molecule analyte to probe the performance of β-CD and has known affinity for the hydrophobic cavity of the sugar. Nitrobenzene, a common plasticizing agent and also a carcinogen, is roughly half the size of fluorescein. Nitrobenzene, being smaller, would be expected to remain in the β-cyclodextrin cavity longer than fluorescein. In many ways, the comparison between the partitioning of the three analytes could be analogous to size exclusion chromatography and could potentially be used to sort analytes by size. Unexpectedly, the fluorescein had a negative signal within the gel; if the fluorescein had
localized within the gel at all, it would have been expected that its presence would have increased the local refractive index compared to salt. However, it can be seen that the signal is less negative for the β-cyclodextrin modified gels, implying that the fluorescein at least localized within the sugar’s cavity and increased the local refractive index temporarily. In regards to nitrobenzene, the responses are positive for both the unmodified and modified gels, but it is noticeably greater (~80 pm shift compared to ~30 pm shift) in the modified gels, again implying the small molecule is interacting with the sugar. By modifying the PAA gels with β-cyclodextrin, it is possible to affect the transport of analyte molecules through the gel, compared to unmodified gels.¹⁰⁶–¹⁰⁸

The modification of the PAA gel through EDC coupling with 2-aminophenylboronic acid (PBA) was also investigated; PBA-modified gels find use as glucose sensors, and so several concentrations of glucose were exposed to the modified gels in increasing and then decreasing concentration.

**Figure 5.6: Surface response of PBA-modified PAA gels to glucose**
A). Resonance wavelength shifts measured for unmodified PAA gels to 0-30 mM glucose in 10 mM steps, with pH 7.4 PBS buffer rinses in between each step. The highlighted region represents the relative spread in response from four microring resonators.

B). Resonance wavelength shifts measured for PBA-modified PAA gels to 0-30 mM glucose in 10 mM steps. The highlighted region represents the relative spread in response from four microring resonators.

Though there is quite a bit of variance in response, it is clear that the responses between the modified and unmodified surfaces are insignificant. This is possibly because of an over-saturation issue; the concentrations used here are in a clinically relevant range and have been used in other literature, yet the gels investigated here are much thinner than commonly probed (at least an order of magnitude thinner). Glucose concentration is important in gauging surface response; if saturated, the gel will swell, as there is 1:1 complexation of glucose with each PBA moiety. If there is not enough glucose to saturated the gel, however, a 1:2 complexation of glucose will occur, with two PBA moieties each complexing to one glucose molecule.\textsuperscript{87,88} This induces gel shrinking instead of gel swelling, as the glucose draws the network in on itself. As gel collapse is easier to interpret on the detection platform (resulting in an increase of local effective refractive index, as there is a higher organic concentration within the sensing region when water is excluded by the shrinking polymer network), perhaps much lower concentrations should be investigated.

Poly(acrylic acid) is environmentally sensitive and easy to chemically modify, but it is not the only hydrogel. Poly(acrylamide) is even more common (and frequently mixed with
PAA), and also extremely sensitive to environmental changes, particularly in solvent. When exposed to an organic solvent (so, not water), the gel will collapse, rapidly de-swelling (See Figure 5.7).

**Figure 5.7: Volumetric changes of PAAm when exposed to different solvents**

A). Real time resonance wavelength shifts of PAAm gels under isopropanol being exposed to increasing amounts of water (in 10% steps). The response of unmodified ring resonators is included for comparison. Data collected by Zachary Weirsma.

B). Real time resonance wavelength shifts of PAAm gels (two sets) under water being exposed to increasing amounts of isopropanol (in 10% steps). The response of unmodified ring resonators is included for comparison.

Though the response of the gels to alternating isopropanol and water is very intense, the gels are uneven across the surface (the two channels, which are separated by less than a millimeter, have noticeably different signals), and the signals do not return to baseline, indicating that the gels never fully desolvate between solvent cycles. However, volume changes
accompanying the introduction of water to the system are still clearly discernable. Observing and characterizing this swelling and collapse on-chip in real time will be insightful for characteristics such as swelling/de-swelling kinetics and future transport studies because the rate of transport is dependent on diffusion though the hydrogel.

5.5 Conclusions

In conclusion, PAAm and PAA gels were successfully grown off the microring resonator surfaces. Synthesis is typically a straight-forward free radical polymerization involving a vinyl monomer and a small percent of crosslinker; the concentration of both controls the brittleness and strength of the resulting gel. These gels can be regionally modified using microfluidics, allowing side-by-side comparisons of chemical and material properties. In addition, the natural sensitivity of the gels to environmental changes (i.e. dehydration, pH changes, and temperature (not shown above)) implies that controlled volume changes could be used to increase sensitivity of the platform to specific analytes. Fine-tuning the responsiveness of the gels is still underway.
Chapter 6: Conclusions and Future Directions

6.1 Conclusions from SI-ATRP-Modified Arrays

Surface-initiated atom-transfer radical polymerization was used to precisely modify the silicon dioxide surface of microring resonator arrays. Several types of polymer brushes, including hydrophilic, hydrophobic, pH-responsive, and thermoresponsive brushes, were grown off the ring surface and characterized using a variety of methods. Spectroscopic ellipsometry was the preferred analysis technique for surface-bound polymer, and analogous growth of free polymer was analyzed primarily by gel permeation chromatography. As is the hallmark of controlled radical polymerization, polydispersity was low for the polymers grown, with length differences limited to 5% on surface.

These brushes, once grown and characterized, were used to enhance analyte interaction with the active sensing region on the detection array. These microring resonators are incredibly sensitive, but this sensitivity decays exponentially with distance from the surface. By organically modifying the ring resonators, analytes in the aqueous mobile phase are encouraged to interact more strongly with the sensing region, compared to an unmodified surface. This was investigated using several analytes of interest, including pesticides, carcinogens, drugs, and chemical warfare mimics. Up to 1000-fold enhancement was observed for certain combinations of analytes and polymer brushes when compared to bare silicon dioxide rings.

The fundamental properties of these thin brushes were then investigated more thoroughly by exposing polymer brushes to plasticizing agents and combinations of solvents/non-solvents. As the brushes are surface-tethered and quite thin, their response to the plasticizing agents differs quite tremendously from their bulk counterparts, and the brush dynamics were highly complex.
Nevertheless, interesting trends emerged, with both length and solubility parameters being dominant factors in brush response. An internal length calibration, relying on the refractive index difference between water and heavy water, was also conducted, and it was found that without more information on the swelling behavior of hydrophilic polymers, this calibration worked effectively for hydrophobic polymers only.

Surface-initiated atom transfer radical polymerization is an excellent way to modify the ring resonators as it allows for precise structural control and thin, highly stable and covalently bound polymer brushes, all of which is necessary when dealing with such a confined sensing region. It is highly versatile and can be used to lend the ring resonators several different chemical functionalities. The interactions between the analytes and the polymer brushes are complex and depend on several factors, including brush length, analyte refractive index, analyte size, analyte solubility in brush, analyte solubility in solvent, and brush solubility in solvent, to name a few.

6.2 Conclusions from Hydrogel-Modified Arrays

Poly(acrylamide) and poly(acrylic acid) hydrogels were also formed on the microring resonator surface, all tethered to the surface using covalent binding to a methacrylate silane on the ring resonators. Owing to the high liquid content and the free radical polymerization method, these gelled surfaces had greater polydispersity than the polymer brush surfaces, but were able to be made reproducibly. Thickness was determined for the dried gels using spectroscopic ellipsometry and was found to be ~20 nm in most cases. The thinness of the dried gels ensured the swollen gels did not extend far past the active sensing region.

Hydrogels are known for undergoing dramatic volumetric changes when exposed to external stimulus such as solvent change, temperature change, or pH change, while allowing for
easy diffusion through the polymer network while swollen. Previously used for directed transport and fluorescent detection, by preliminarily investigating the integration of gels with rings, the feasibility of on-chip directed transport was assessed for sample concentration and improved sensitivity.

Poly(acrylamide) is one of the best-characterized hydrogels of all time and was used as the proof-of-concept hydrogel before moving onto more scientifically interesting gels. A 90% PAAm gel was made by radiation-initiated free radical polymerization of a monomeric solution drop cast on a silanized ring resonator array and then exposed to increasing concentrations of water from isopropanol. As PAAm does not swell in IPA, the swelling of the gel with water exposure was able to be tracked in real time, indicating that the ring resonator platform was appropriate to monitor real time volumetric changes of gels.

Poly(acrylic acid) is the acidified form of PAAm, and can in fact be derived from the PAAm gel directly if desired. Considering this requires chemicals slightly too harsh for the resonator platform, PAA was made independent of PAAm, by another free radical polymerization. Besides being pH sensitive owing to the carboxylic acid functional group, PAA is also easy to functionalize, and was modified with phenylboronic acid and β-cyclodextrin pendant groups. PBA is commonly used in glucose detection as it complexes in either 1:1 or 1:2 with glucose, and β-cyclodextrin has been used in host-guest chemistry as it has a hydrophilic outer shell but a hydrophilic inner cavity, ideal for trapping organic molecules in aqueous solutions. Unmodified PAA was used for the detection of pH and ionic strength changes, with the gel swelling in basic and high ionic strength conditions and collapsing in acidic and low ionic strength conditions.
6.3 Future Directions: Polymer Brushes

The growth of polymer brushes off the microring surface now being well-understood and implanted, and having seen that the desired effect of signal enhancement is indeed achieved, it is obvious that next steps center around increasing the selectivity of the enhancements. As it stands, though signals are tremendously enhanced when compared to unmodified surfaces, these enhancements have been unpredictable and difficult to interpret (why glyphosate has such low enhancement compared to the other organophosphate, 4-methylumbelliferylphosphate, for example, or why p(NIPAM) enhances acetaminophen in water but not Bisphenol A in water). Obviously there is a combination of factors at work, but de-convoluting these from each other is not straightforward, and microring resonators cannot be relied upon as the only method.

Microring resonators, being bulk property detectors, have lent the great power to detect non-chromophoric and non-fluorogenic species in low concentration, exactly as desired, but leave a reasonable amount of mystery as to exactly how these enhancements occurred. Did the brush swell in the solvent and the signal is now merely greater because more of the sensing region is occupied? Did the brush collapse and trap the analyte close to the ring surface? Is the brush becoming less crystalline with exposure to the analyte, allowing for faster and greater diffusion? Separating these physical processes from one another cannot merely be performed on the ring resonators, and liquid cell ellipsometry, liquid cell AFM, and perhaps even differential scanning calorimetry could be useful in understanding what is happening on the surface, by probing brush thickness, brush elasticity, and glass transition temperature (although these measurements are of course notoriously finicky).61,109
On one hand, then, the fundamental physical knowledge of the system must be expanded upon. On another, though, selectivity of the brushes to specific analytes can be controlled chemically, and should be investigated as well. There are many reactive brushes, such as Q-poly(METAf), which reacts with the organophosphate group of pesticides and nerve agents and has previously been integrated with other detection motifs.\textsuperscript{110} Charged brushes, such as zwitterionic poly(sulfobetaine methacrylate) or cationic poly(L-lysine)-graft-poly(ethylene glycol)\textsuperscript{46,111} obviously have strong interactions with oppositely charged analytes, and could potentially be used to separate mixtures of analytes on the ring array. Other interactions, such as pi-pi stacking between aromatic p(St) and aromatic analytes such as Bisphenol A or nitrobenzene, or hydrogen bonding gradients could also be used to enhance selectivity between certain analytes and the polymer brush. Forming gradients of brushes, in length, branching, or composition, would also be a strategic next step in turning to the ultimate goal of integrating analyte separation, concentration, and detection onto a single device.\textsuperscript{32}

6.4 Future Directions: Hydrogels

There is great opportunity with ring-adhered hydrogels, which has been only briefly touched on in the most preliminary way so far. The very basic experiments conducted herein take advantage of only the tip of the exciting possibilities in functionalization and molecular imprinting available with gels, not to mention having been limited solely to water-swollen (hydrogels). Organogels, swollen by organic solvents such as dimethylsulfoxide or tetrahydrofuran, are also an important polymer class and may have even greater field applicability in analyte capture and transport, as these gels do not suffer from the same dehydration issues as hydrogels experience in ambient conditions.\textsuperscript{112,113}
Chemical modification was also only addressed in a very basic way – hydrogels are straightforward to modify, and patterning the gels to embed gradients as previously discussed could allow for separation, concentration, and detection of analytes to occur on a single platform! Here, only spotting and limited microfluidics were used to selectively modify regions of the gel, but photo-patterning, stamping, or more complex microfluidics could also be used to incorporate several gradients on one gel.

The use of p(NIPAM) hydrogels was not introduced although some cursory studies have been conducted; these gels are incredibly powerful in their thermoresponsiveness.\textsuperscript{42,92,114} Coupling environmentally-stimulated gels, which undergo dramatic phase changes when exposed to their stimulus, with the ring resonators has great potential for tremendous sensitivity enhancements. Indeed, pH-sensitive gels were discussed, but p(NIPAM) in particular has a dramatic volumetric collapse when the temperature is raised above the length-dependent lower critical solution temperature. It is easy to envision tracking an exothermic reaction on-chip, or cycling heating and cooling cycles to trap analytes close to the sensing region and then release them for regular sampling.

Molecular imprinting is one other avenue of interest;\textsuperscript{3,115} the idea of incorporating specifically-shaped holes within a gel matrix such that analytes diffusing through the gel get trapped according to their functionality has been investigated in several iterations, often failing because of imprint collapse, imprint diffusion, or lack of signal transduction. This is clearly quite a complex problem, but by tuning the gel crystallinity and making gradients of holes (gradients of number of holes, instead of gradients of holes of differing chemical functionality), this opens another route for selective and sensitive measurements.
6.5 Concluding Remarks

Surface-tethered organic modifications of a whispering gallery mode sensor array improved sensor sensitivity and selectivity. This technology shows promise for field applications in the real-time detection of toxins and pesticides, in addition to being a novel approach for studying fundamental properties of polymers, including diffusion, relaxation, and possibly transport. Integrating the silicon photonic microring resonators with selectively reactive surfaces, or interfacing with other separation/detection platforms, including but not limited to HPLC, SEC, or fluorescence, would further enhance the selectivity and sensitivity of this platform.
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