DIENE CONTAINING POLYMER BRUSHES FOR BIOIMMOBILIZATION

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To my dear family

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ABSTRACT

DIENE CONTAINING POLYMER BRUSHES FOR BIOIMMOBILIZATION

Reactive polymer brushes have emerged as an active area of research in recent years. The increasing popularity of polymer brushes is due to the multiple advantages that they offer. In fact, they can be obtained with straightforward technical synthesis and they are suitable to a large range of functionalizable molecules. Compared to the polymers coated on a substrate with spin-coating technique, they are very resistant to harsh chemical environment. Surface-initiated atom transfer radical polymerization (ATRP) is commonly utilized since it provides good control of the polymer brush composition and thickness. Over the past decade, efficient chemical transformations based on various “click” reactions have been employed for functionalization of polymeric brushes. To date, all approaches require additional reagents and/or catalysts to achieve attachment of desired molecules. This thesis reports the first example of utilization of a Diels-Alder reaction based on functionalization strategy that allows efficient conjugation of polymer brushes under mild and reagent-free conditions. Polymer chains incorporating furan groups as side chains are “grafted from” silicon surfaces. These pendant furan moities react with a maleimide functionalized molecule of interest through the Diels-Alder reaction. The specificity of the functionalization is gained by the presence of the antibiofouling polyethylene glycol methyl ether methacrylate monomer. Copolymers with varying amounts of furfuryl methacrylate, were synthesized in this study. The reactivities of these furan containing brushes were investigated through the immobilization of maleimide functionalized molecules, such as the model compound N-ethylmaleimide, a fluorescent dye BODIPY-maleimide and biotin-maleimide ligand which enables the immobilization of Quantum-Dot streptavidin conjugates. Additionally, the reversibility of these conjugations is demonstrated by taking advantage of the reversible nature of the Diels-Alder reaction.

ÖZET

BİYOSABİTLEME İÇİN
DİEN İÇEREN POLİMER FIRÇALAR

Reaktif polimer fırçaları son yıllarda ilgi çeken bir araştırma alanına dönüşmüştür. Polimer fırçalarına olan artan rağbetin başlıca sebebi, sundukları çok sayıda avantajlardır. Ayrıca, basit sentez metotlarıyla elde edilebilmektedirler ve birçok fonksiyonelleştirilebilir gruplarla uyumludurlar. Yüzey kaplama yöntemiyle elde edilen polimer kaplı filmlere kıyasla, zorlu kimyasal ortamlara daha dayanıklılardır. Kompozisyonları ve kalınlığının kontrolü iyi bir şekilde sağlandığı için, polimer fırçalar sıkça yüzeyde başlatılan atom transfer radikal polimerizasyon (SI-ATRP) tekniğiyle hazırlanmaktadır. Geçtiğimiz son on yılda, çeşitli ‘klik’ tepkimelerine dayananan etkili kimyasal dönüşümler polimer fırçaların fonksiyonelleştirilmesinde kullanılmaktadır. Günümüze kadar, istenilen molekülün başarıyla bağlanması için tüm yaklaşımlar ekstra ajanların ve/veya katalistlerin kullanımını gerektirmekteydi. Bu tez, polimer fırçaların ılımlı ve ajan gerektirmeyen koşullarda etkili konjugasyonuna olanak sağlayan Diels-Alder tabanlı fonksiyonelleştirme stratejisinin kullanımının ilk örneğini rapor etmektedir. Yan dallarında furan grubu barındıran silikon yüzeyler üzerinden büyütülmüştür. Yan dallardaki furan grupları maleimide fonksiyonelliği olan moleküllerle Diels-Alder tepkimesi ile tepkime vermektedir. Bu tepkimenin spesifik olması istenmeyen bağlanmayı engelleyen polietilen glikol metil eter metakrilat monomerinin varlığıyla sağlanmıştır. Çalışmada çeşitli miktarlarda furfuril metakrilat içeren kopolimerler sentezlenmiştir. Furan içeren polimer fırçaların reaktifliği maleimid fonksiyonelliği olan moleküllerle, örneğin model bileşik N-etilmaleimid, floresan boya olan BODIPY-maleimid ve Quantum-Dot streptavidin konjugatının sabitlenmesine izin veren biyotin maleimid ligandı ile incelenmiştir. Ek olarak, bu konjugasyonların geri dönüşümlülüğü, Diels-Alder tepkimesinin geri dönüşümlü olması özelliğinden yararlanılarak gösterilmiştir.

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LIST OF ACRONYMS/ABBREVIATIONS

J	Coupling constant
AFM	Atomic Force Microscopy
ATR	Attenuated Total Reflectance
ATRP	Atom Transfer Radical Polymerization
BODIPY	Boron-dipyrromethene
BSA	Bovine serum albumin
$CDCl_3$	Deuterated Chloroform
CH_2Cl_2	Dichloromethane
DA	Diels-Alder
DMAP	4-(dimethylamino)pyridine
DSC	Disuccinimidyl carbonate
EDC	1-ethyl-3-[3-dimethylaminopropyl]carbodiimide
Et_2O	Diethyl ether
FT-IR	Fourier Transform Infrared
FuMA	Furfuryl methacrylate
GPC	Gel Permeation Chromatography
MeOH	Methanol
NHS	N-hydroxysuccinimide
NMR	Nuclear Magnetic Resonance
PEGMEMA	Poly(ethylene glycol) methyl ether metahcrylate
PHEMA	Poly(2-hydroxyethyl methacrylate
PMDETA	N,N,N',N',N''-Pentamethyldiethylenetriamine
POEGMA	Poly(oligo(ethylene glycol) methacrylate
Q-Dots	Quantum-Dots
rDA	Retro Diels-Alder
TEA	Triethylamine
THF	Tetrahydrofuran
UV	Ultraviolet
XPS	X-Ray Photoelectron Spectroscopy

1. INTRODUCTION

1.1. Historical Background

The concept of polymer brushes was first described by Alexander in 1977 [1], de Gennes in 1980 [2], and Semenow in 1985 [3] with the development of accurate theoretical studies of polymer brushes. Before that, there were some publications about polymer brushes at the experimental level, without in-depth analysis, due to the lack of techniques concerning the characterization and identification of these tethered layers [4]. For many years research about polymer surfaces remained to the domain of engineering and materials science [5-6]. New techniques of polymerization introducing facile synthesis routes for the design of macromolecules promoted the usage of polymer chemistry in this area [7-8]. Advancements in synthesis of polymer brushes in the 1990s allowed their fabrication with precise control of their molecular weight and brush height, and the field started to benefit from their properties to target specific applications. This evolution promoted the spreading of application of polymer brushes to the field of biology and physics [9].

Although polymer brushes concept have been investigated by researchers for the past 20 years [10-11], applications of polymer brushes were very restricted in terms of the reactivity of the polymeric surfaces with exceptional mechanical and chemical properties. Progressively, the controllable characteristics of the polymer chains grafted on a surface extended their popularity and become a successful domain of research. In fact, the properties of polymer brushes can be tailored for a specific application; the composition, molecular weight, grafting density, thickness and architecture of the polymeric layers can be adjustable precisely for the expected application, without using complicated synthesis methods. Nowadays, polymer brushes have a major application particularly in nanotechnology as “nanoarchitectonics” with biocompatibility, redox activity, potentiality of energy storage and photophysical properties [12-13]. Additionally, lithographic methods allow the initiation of polymer brush as surfaces presenting tailored patterned areas [14-15].

1.2. General Features of Polymer Brushes

1.2.1. Definition

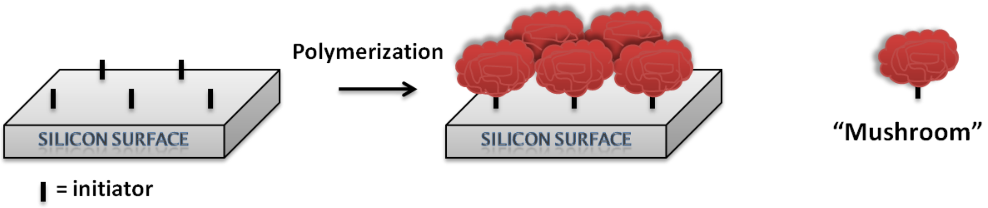
Polymer brushes are defined as a group of polymer chains that are attached by one of their extremities to a surface with a certain density of adhesion points [16]. At sufficiently high grafting density, steric repulsions occur between these polymer chains during their growth from the surface. As a consequence of their strong steric repulsions, the polymer chains are presented in a defined orientation; perpendicular to the surface, and they adopt a "brush" conformation. Conformation of polymer chains grafted on a surface is very different from "free" polymeric chains in solution. Overall, they differ not only in their configurations but, as a result, also in their properties and applications.

1.2.2. Polymer Brushes Properties

Polymer chains grafted onto a surface can adopt "mushroom" or "brush" conformations (Figure 1.1). This structural difference is due to the difference in the "grafting density" of the polymer brushes. For the polymers synthesized through surface-initiated methods, grafting density is determined by the grafting density of initiators. If the grafting density of the initiators is low, polymer chains will grow like a "mushroom" (Figure 1.1a). They have enough space that they can occupy without steric hindrance. If the grafting density of the initiators is high, polymer chains will grow in the shape of a "brush" (Figure 1.1b). In this case, the space between each polymer chain is restricted, so repulsive interactions take place between them and they are forced to extend perpendicular to the surface.

Polymer brushes growing perpendicularly to the surface have different properties compared to polymers in solution. This gives novel properties to polymeric brushes, such as control of flocculation, wetting and diffusion, control of phase-segregation, regulation of steric repulsion, interfacial disposition of end groups, control of bio-molecular immobilization and lubrication [4].

a) **Low Grafting Density**



b) **High Grafting Density**

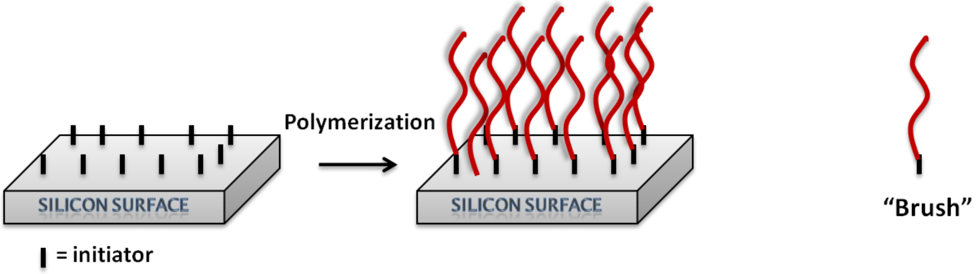


Figure 1.1. (a) Conformation of polymer brush with low grafting density. (b) Conformation of polymer brush with high grafting density.

1.3. Growth of Surface-Grafted Polymer Brushes

End-grafted polymeric films are defined as thin polymeric surfaces conceived by the attachment of long polymer chains at one extremity onto a surface through covalent interactions. These interactions between the polymeric chains and the surface are very strong and it is advantageous comparing to other polymeric surfaces prepared using various techniques such as spray coating and doctor blading, dip coating and spin coating [17]. In fact, this strong covalent attachment gives resistance to the polymeric surface towards harsh chemical conditions and high temperatures.

There are different methods to synthesize polymer brush surfaces and the method chosen to synthesize them is determinant for the conformation of the polymeric backbone. As a matter of fact, according to the synthetic routes used, they can adopt the “mushroom” or extended “brush” conformations. Commonly, there are two methods utilized to obtain these polymeric coatings; “grafting to” and the “grafting from” method.

1.3.1. “Grafting to” Method

“Grafting to” method is commonly used to obtain polymer brushes coated on surfaces. This method consists of the attachment of preformed polymer chains containing appropriate reactive functional end groups with a suitable substrate (Figure 1.2). This is realized in liquid medium in order to facilitate the attachment of the polymer chains onto the substrate. The covalent liaison between the surface and the polymers gives resistance and robustness against environmental conditions. Generally, reactive groups that are used as anchoring units are silanes, acids and thiols. The main disadvantage of this method is the low grafting density obtained due to the difficulty to make a large polymer and a surface react together. Also, this method necessitates certain compatibility between the different chemical structures making the polymeric surface.

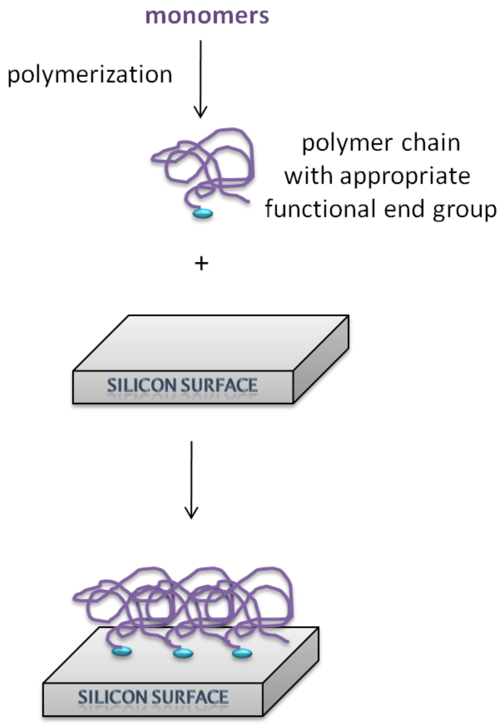


Figure 1.2. Synthesis of polymer brushes with “grafting to” method.

Generally, with the “grafting to” method, only a little amount of polymer can be attached to the surface. As the thickness of the grafted polymeric film increases, there is a barrier established by the already attached polymer chains, so it becomes very demanding for the macromolecular chains to reach the surface. This restriction explains why the grafting density and the thickness of the polymer brushes synthesized with “grafting to” method are low compare to these synthesized with “grafting from” method. In recent years, the later method has attracted a lot of attention in the preparation of polymer brushes coated on surfaces with high grafting density and high thickness [18].

1.3.2. “Grafting from” Method

The “grafting from” method is employed in this research to obtain functional polymer brushes. This method synthesis is composed of two steps; the first step consists of the immobilization of initiator molecules on the silicon surface, and the second step consists of the polymerization that occurs from the surface after adding the monomers in the reaction medium in the presence of an appropriate catalyst (Figure 1.3).

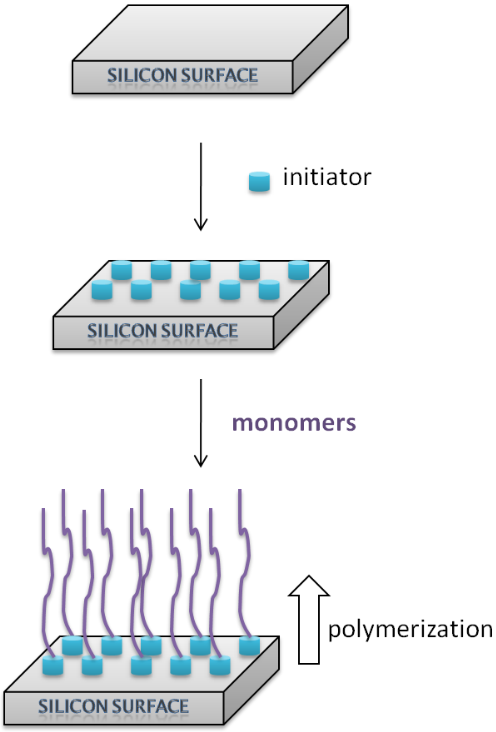


Figure 1.3. Synthesis of polymer brushes with “grafting from” method.

In the first step, the immobilized initiator molecules form a monolayer and the polymerization starts exclusively from the surface, thus this technique is termed as “surface-initiated polymerization” (SIP). Generally, the immobilization of the initiators is obtained through the reaction between acid bromides or acid chlorides and surface amino/hydroxyl groups. If the initiators are densely immobilized to the surface, the grafting density will be high. Thus, the density of the initiators determines the density of the polymer chains and can be adjusted to obtain desired thickness [19]. As mentioned before, this method of polymer brushes synthesis gives higher grafting density and so thicker polymer brush films are obtained as compared to the “grafting to” method (Figure 1.4). The tunable, controllable and versatile characters of this synthetic method, makes it an attractive approach. It can be applied in many research fields, such as nanocomposites, adhesive materials, chromatography, colloid stabilization and bio-mimetic surfaces [17].

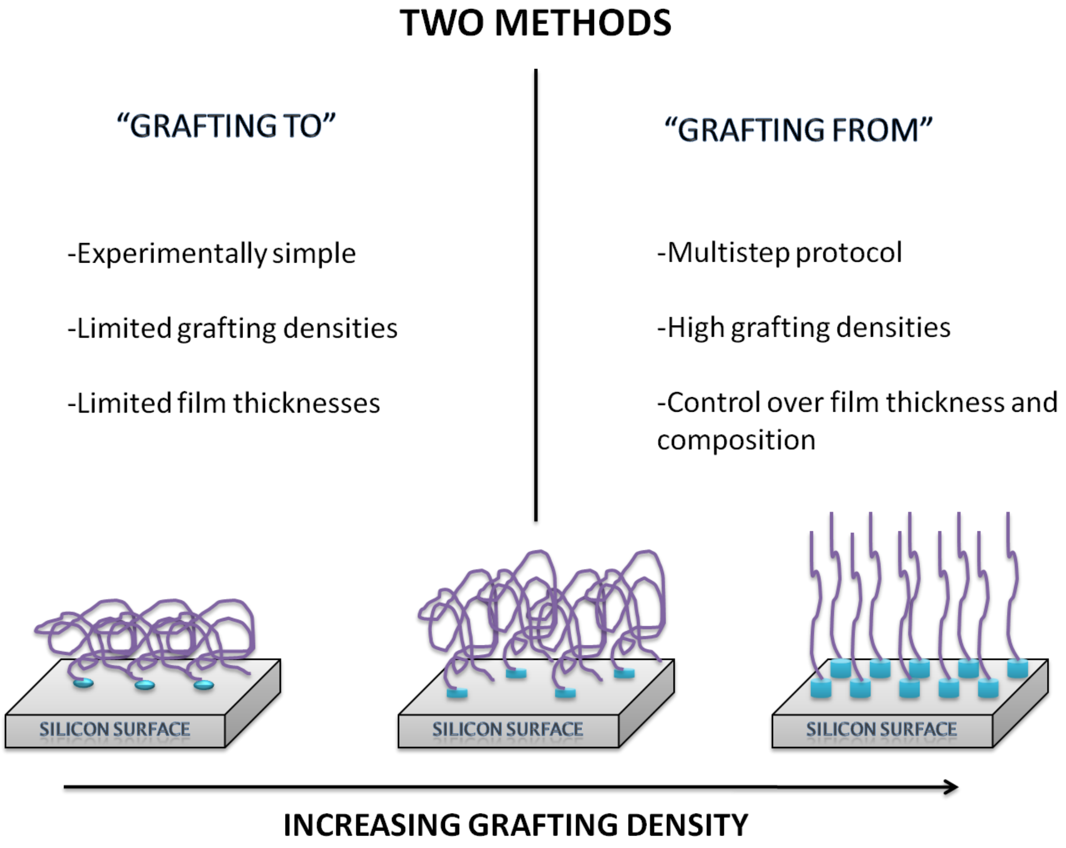


Figure 1.4. General aspects of the two principal methods in the synthesis of polymer brushes.

1.4. Atom Transfer Radical Polymerization

To date, polymer brushes have been synthesized using various polymerization techniques such as ring-opening metathesis polymerization (ROMP), reversible addition fragmentation chain transfer (RAFT), nitroxide mediated polymerization, living anionic or cationic polymerization, atom transfer radical polymerization (ATRP) and living ring-opening polymerization [20].

ATRP, the method utilized in this study, is a polymerization technique discovered in 1995 by Krzysztof Matyjaszewski and Mitsuo Sawamoto. ATRP is a controlled/ “living” radical polymerization (CRP) technique which allows a precise control over the architecture, functionality and composition of the macromolecules. This aspect of ATRP makes it an important method in order to synthesize functional polymers for a specific application [21]. Another advantage of this polymerization technique is its compatibility with a wide range of vinyl monomers such as acrylates, acrylamides, styrenes and acrylonitriles [22].

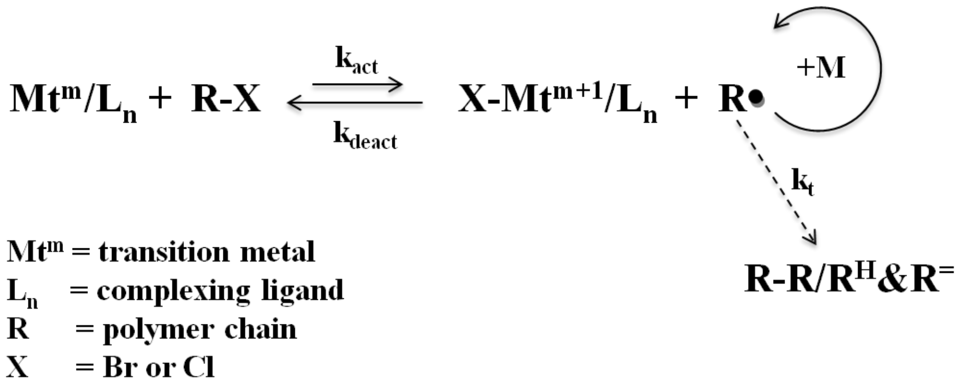


Figure 1.5. Simplified mechanism of ATRP.

ATRP mechanism consist of the equilibrium between two principal actors; the active propagating form ($R^{\cdot}$) present in low amount and the dormant species (R-X) present in higher amount (Figure 1.5). A transition metal complex (Mt^m/L_n) is formed by a ligand and a metal like copper or iron and catalyzes the reversible redox reaction to form the radicals, which react with the monomers to initiate the polymerization. The termination step takes place via disproportionation or radical coupling, but only a small quantity of polymers undergo the termination. The termination step is minimized by the reduced concentration of active radicals compare to the dormant forms of the polymer chains present in higher concentration [21].

1.4.1. Surface-Initiated ATRP

Surface-initiated ATRP is a very powerful method for the synthesis of polymer brushes due to the many advantageous of ATRP. In fact, the polymerization can occur in mild conditions, in aqueous environments and at room temperature. Since ATRP is a controlled/ “living” polymerization, the thickness of the polymeric brush can be adjusted by varying the concentration of monomer, the grafting density of the initiators, as well as the duration of polymerization [22].

Ruehe et al. demonstrated that free-radical polymerization is an attractive way in order to produce polymer chains attached covalently onto a surface with high grafting densities [23]. The number of initiator molecules immobilized on the surfaces determines the number of the grafted polymer chains. This feature can be utilized to control the grafting density of the polymeric brushes.

In order to obtain flat polymer brushes, silicon surfaces are the most widely used substrates for surface initiated-ATRP. The ATRP initiators can be immobilized to the surface through organosilanes or strong Si-C bonds. Si-H surfaces are preferentially used instead of Si/SiO_2 wafers in the synthesis of polymeric films with stable Si-C bonds. After exposure of Si-H surfaces under oxygen plasma, ATRP initiator molecules are immobilized on the surfaces.

Furthermore, ATRP allows the functionalization of the substrates in a very simple way using benzyl halides or α-haloesters that are commercially available [21]. Since the initiator molecules can be effectively coated on these surfaces, polymer chains can be grown as spherical particles or flat surfaces (Figure 1.6).

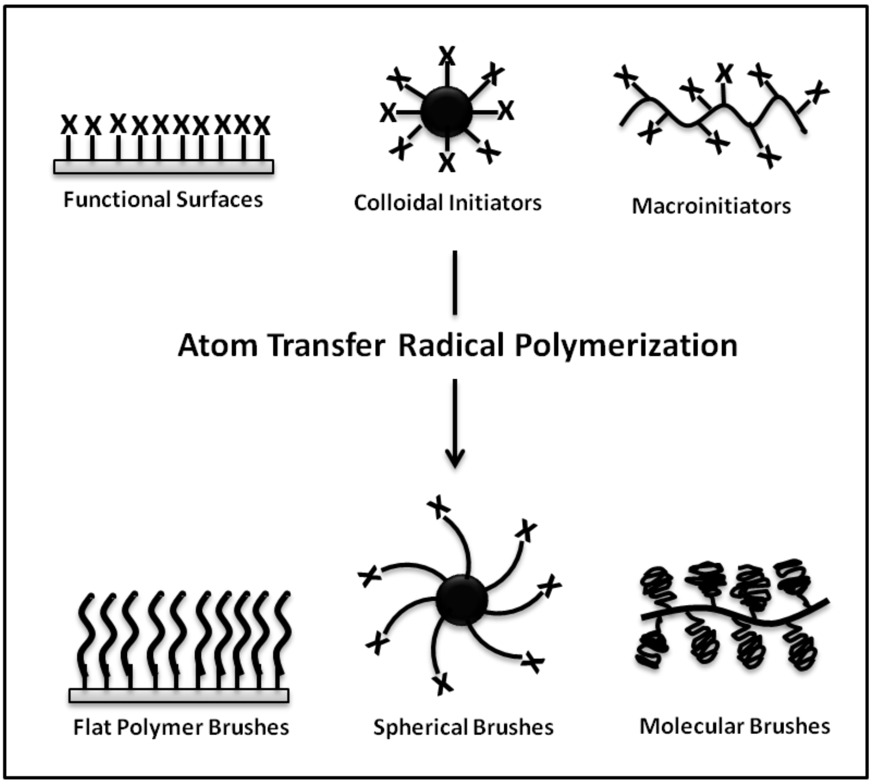


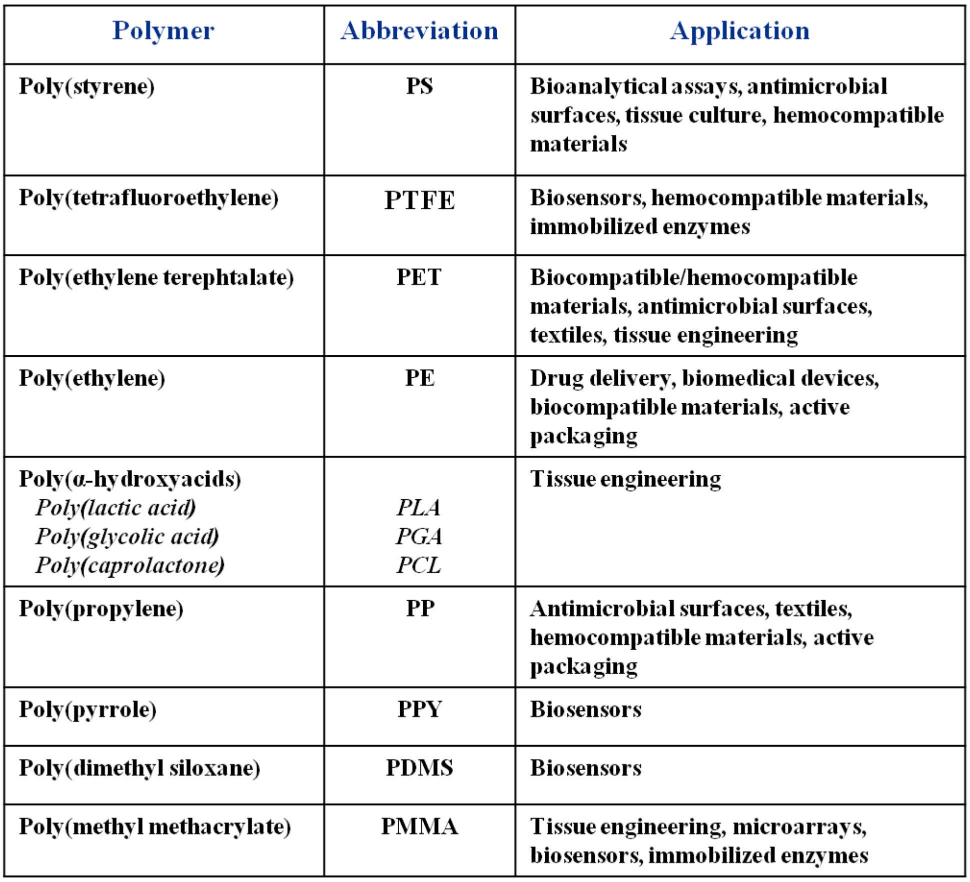
Figure 1.6. Polymer brushes synthesized with surface-initiated ATRP from flat surfaces colloids and polymers (X, halogen).

1.5. Reactive Surfaces Based on Polymer Brushes

Immobilization of bioactive molecules on polymer films has been very attractive in the last years in bioprocessing, textiles, food packaging, biomedical, microelectronics and as drug delivery systems (Table 1.1) [24].

Polymer brush surfaces are veritable candidates for protein immobilization, enrichment and purification. As a matter of fact, polymer brush surfaces have multiple advantages compare to the other polymer films. First of all, they abound in functional groups in their structures that can be derivatized to change the properties of the surfaces for a specific application. Moreover, the abundance of functional reactive groups and their three-dimensional conformation allow improved binding capacity and immobilization than self-assembled monolayers (SAMs) [25].

Table 1.1. Applications of polymer substrates.



Polymer	Abbreviation	Application
Poly(styrene)	**PS**	**Bioanalytical assays, antimicrobial surfaces, tissue culture, hemocompatible materials**
Poly(tetrafluoroethylene)	**PTFE**	**Biosensors, hemocompatible materials, immobilized enzymes**
Poly(ethylene terephtalate)	**PET**	**Biocompatible/hemocompatible materials, antimicrobial surfaces, textiles, tissue engineering**
Poly(ethylene)	**PE**	**Drug delivery, biomedical devices, biocompatible materials, active packaging**
Poly(α-hydroxyacids) *Poly(lactic acid)* *Poly(glycolic acid)* *Poly(caprolactone)*	 *PLA* *PGA* *PCL*	**Tissue engineering**
Poly(propylene)	**PP**	**Antimicrobial surfaces, textiles, hemocompatible materials, active packaging**
Poly(pyrrole)	**PPY**	**Biosensors**
Poly(dimethyl siloxane)	**PDMS**	**Biosensors**
Poly(methyl methacrylate)	**PMMA**	**Tissue engineering, microarrays, biosensors, immobilized enzymes**

An elegant application of polymeric brushes in protein immobilization was reported by Klok and coworkers [26]. For this purpose, they synthesized poly(glycidyl methacrylate)-*co*-poly(2-(diethylamino)ethyl methacrylate brushes via surface-initiated ATRP (Figure 1.7). They compared the protein immobilization capacities of the copolymer-brush-covered chips and of the reference substrate, dodecylphosphate-modified chips; they concluded that immobilization of the proteins is higher for the polymer-brush-coated chips (Figure 1.8). Furthermore, they demonstrated that the protein immobilization increases with the polymer brush thickness. Additionally, they showed that the copolymer brushes have a higher protein binding capacity than the poly(glycidyl methacrylate) homopolymer brush. In fact, the addition of 2-(diethylamino)ethyl methacrylate enhances the epoxide ring-opening reaction of the glycidyl methacrylate thus increasing the immobilization of proteins (Figure 1.8).

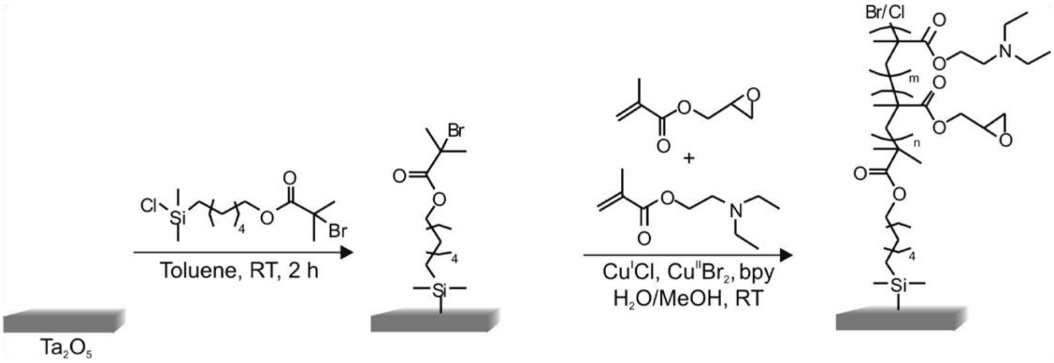


Figure 1.7. Synthesis of the (co)polymer brush [26].

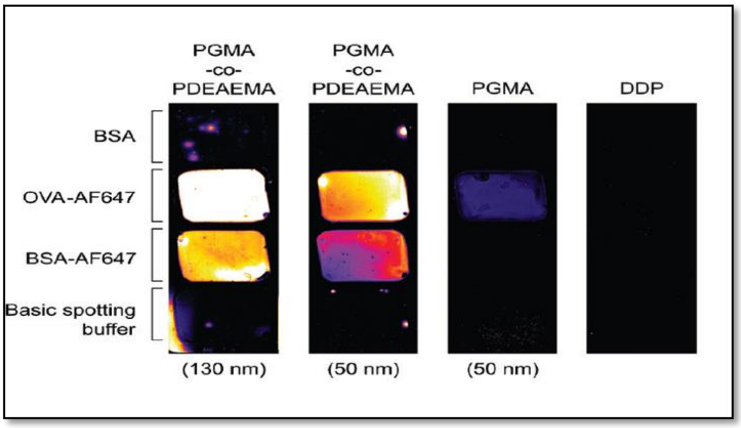


Figure 1.8. Fluorescence images of the different chips [26].

As another example of functionalizable polymer brushes, Patton et al. synthesized brushes containing alkyne functional groups via surface-initiated polymerization [27]. The alkyne bearing brushes enabled attachment of thiol containing biomolecules, such as N-acetyl-L-cysteine or thiocholesterol, through radical-mediated thiol-yne “click” reaction. A dithioether adduct was formed after the reaction of an alkyne with a thiol group (Figure 1.9a). They applied thiol-yne reactions in sequence by using a photomask to obtain patterned multicomponent brush surfaces (Figure 1.9b).

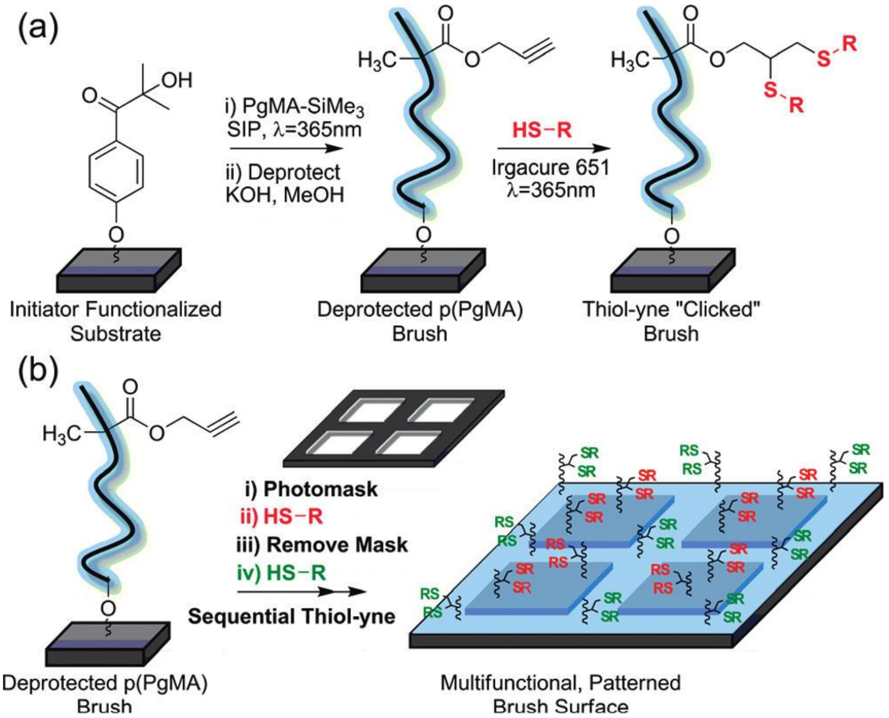


Figure 1.9. (a) Synthesis of thiol-yne “clicked” polymer brushes. (b) Procedure of patterned multicomponent polymer brushes synthesis [27].

Baker and coworkers used a similar synthetic strategy for bioconjugation [28]. They synthesized azidopropyl methacrylate based polymer brushes via surface-initiated ATRP. The pendant azide groups enable their reaction with alkyne functionalized bio-molecules via “click” chemistry. Copolymers of azidopropyl methacrylate and hydrophilic poly(ethylene glycol) methyl ether methacrylate were utilized to increase the yield of bioconjugation in aqueous environment.

A method which is commonly utilized in protein immobilization is the post-polymerization modifications that involves activation of polymer brushes. Bruening et al. modified poly(2-hydroxyethyl methacrylate) (PHEMA) brushes with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) coupling. The ester groups of PHEMA brushes were activated in order to incorporate Cu complex which immobilize bovine serum albumin (BSA) [29]. Ramstedt et al. synthesized poly(oligo(ethylene glycol) methacrylate) (POEGMA) brushes to immobilize histidine-tagged (His-tagged) proteins [30]. The hydroxyl end groups are activated by disuccinimidyl carbonate/4-(dimethylamino)pyridine (DSC/DMAP) coupling. Then, nitrilotriacetic acid (NTA) molecules, reacting with the carbonate functions, provide a handle for the complexation with His-tagged proteins (Figure 1.10).

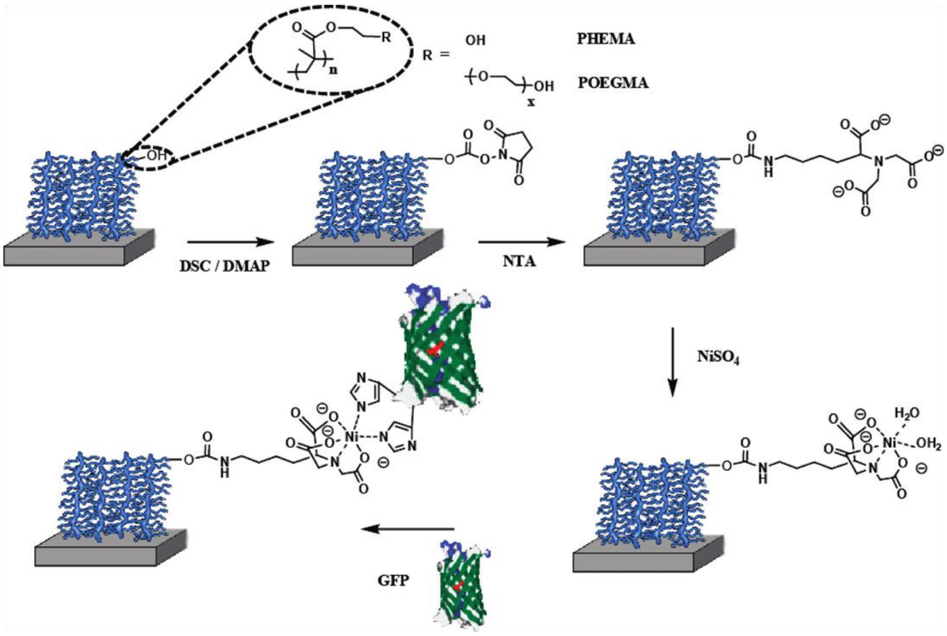


Figure 1.10. Complexation of NTA-functionalized brushes with His-tagged proteins [30].

1.5.1. Polymer Brushes via Photo-Patterning

Polymer brushes synthesized via surface-initiated polymerization are unique structures utilized to develop sensory layers that can be used in different application areas. They are often organized in three-dimensional structure, similarly to two-dimensional self-assembled monolayers; their larger film thickness allows them to become an interesting platform in order to fabricate high-capacity reactive groups binding surfaces [26]. This aspect is very important for biosensor applications [31].

The synthesis of patterned polymeric surfaces with tunable physicochemical properties has been very popular in the past two decades in micro- and nanotechnology thanks to the multiple advantages cited above. Patterned polymer brush surfaces is easily synthesized; with lithography utilizing irradiation or with mechanical force applied locally. This later method is problematic because of the removed material debris [32].

For its simplicity and efficiency, ultraviolet (UV) lithography is widely used. It consists in the transfer of a patterned photographic mask onto a surface. First of all, the surface is coated with a polymeric photosensitive material called as “photoresist”. The surface is then exposed to UV light with a photomask in order to obtain patterning regions (Figure 1.11a). The regions of the photoresist film exposing to the UV light are removed, because they are de-polymerized under UV (Figure 1.11b) [33-34]. The patterned photoresist, which is remaining, protects the film underneath during the etching process. A suitable solvent is then used in order to remove this remaining photoresist (Figure 1.11c).

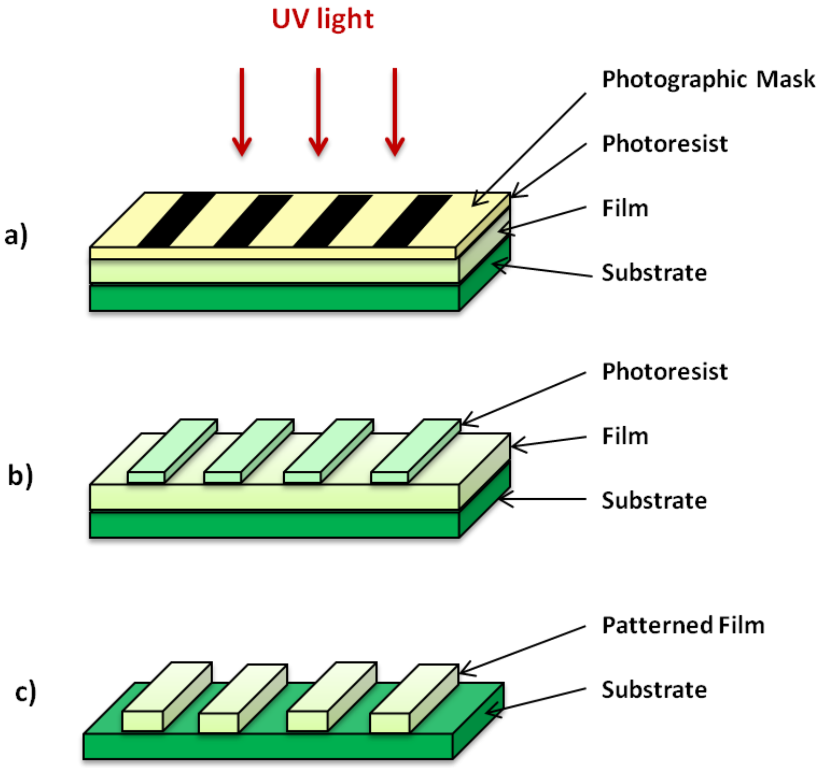


Figure 1.11. Schematic representation of UV Photolithography [34].

In a recent study, Ober and coworkers described a technique for the control of proteins and neuronal cell attachment using patterned polymer brushes [35]. For this purpose, poly(polyethylene glycol methacrylate-*ran*-(2-methacryloyloxy)ethyl-trimethyl ammonium chloride) or poly(PEGMA-*ran*-MAETAC) brushes were synthesized with “grafting from” method (Figure 1.12). The tertiary amine in the polymer backbone mimics acetylcholine as a neurotransmitter, which regulates and promotes neuronal cell development. In fact, at about pH=7, the tertiary amines are protonated, so positively charged and they can obtain similar features to acetylcholine. PEGMA was used for its non-biofouling properties and its biocompatibility.

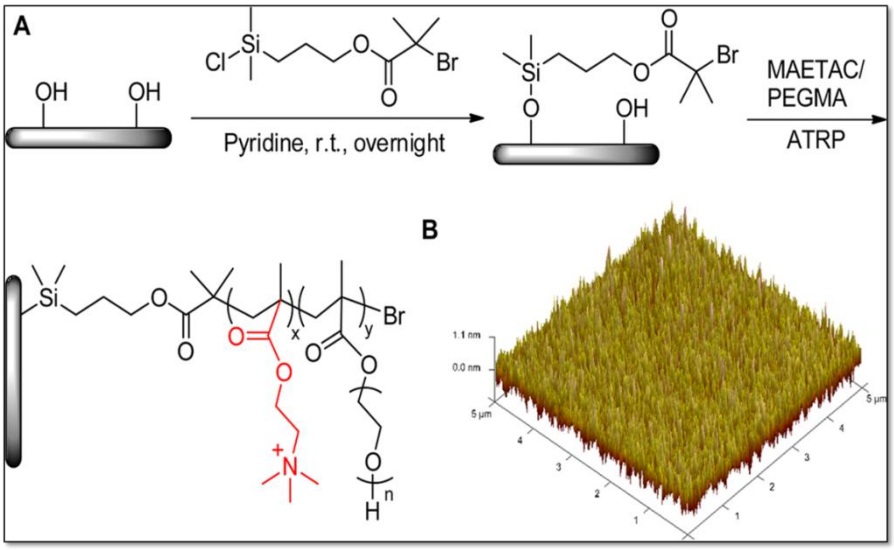


Figure 1.12. Poly(PEGMA-ran-MAETAC) brushes synthesis [35].

Patterned surfaces were fabricated using UV-photolithographic method (Figure 1.13). PEGMA-silane self-assembled monolayer (SAM) provides non-adhesive domains on the surface. The polymer brushes were synthesized from the ATRP initiator containing regions. As shown in Figure 1.13, neuronal cells adhere and grow in the acethylcholine functionalized polymer brush area, while establishing better connections in the broad patterning regions.

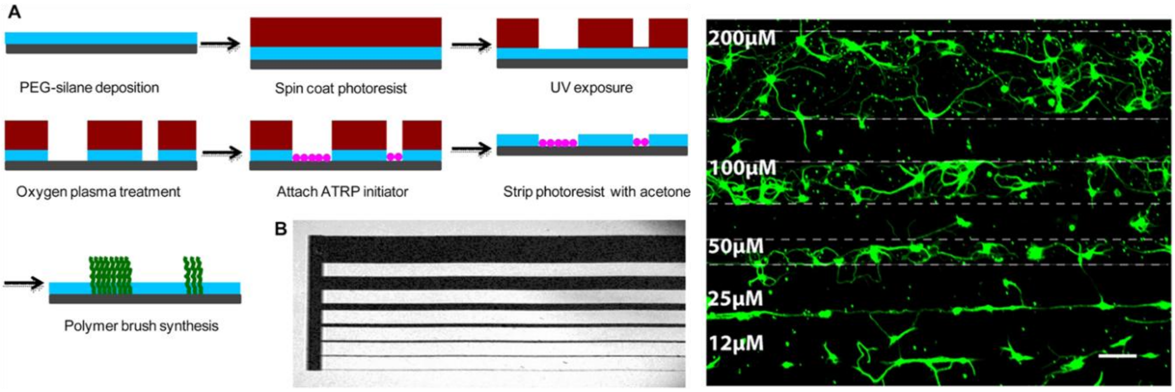


Figure 1.13. Patterned neuronal cells on polymer brush film [35].

Another method to obtain patterned polymeric brushes employs the patterning of surfaces with initiator. Methods such as micro-contact printing of initiators [36] or photo-patterning of initiators by spatially controlled photo-decomposition of initiator coated surface using UV-irradiation [37] can be used.

1.6. Diels-Alder Reaction as a Conjugation Tool

Diels-Alder (DA) reaction is a widely used reaction for the synthesis of organic compounds. In this reaction an electron-rich "diene", reacts with an electron-deficient dienophile through a [4+2] cycloaddition. It is electrocyclic, including the 4 л-electrons of the electron-rich diene and the 2 л-electrons of the electron-poor dienophile. Energetically, the σ-bonds formed in the cyclo-adduct are more stable than the л-bonds of the two reactants. Generally, the Diels-Alder reaction provides products in high yields under mild conditions without formation of unwanted side-products.

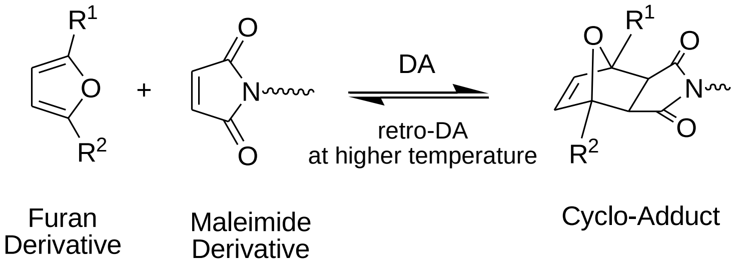


Figure 1.14. Thermoreversible Diels-Alder reaction between furan derivate and maleimide derivative.

Furan and anthracene molecules are frequently chosen as diene components and maleimide is widely used as a dienophile (Figure 1.14). Among the many advantages of Diels-Alder reaction, its thermoreversibility is quite relevant. For example, when furan is used as a diene, Diels-Alder reaction takes place up to 80 °C, but above about 110 ^{o}C the reverse reaction, the retro-Diels-Alder (rDA) reaction dominates [38].

1.6.1. Diels-Alder Reaction in the Synthesis of Reactive Polymeric Coatings

Previously, our group reported the synthesis of reactive thin polymeric films containing furfuryl groups [39]. Bio-molecules were immobilized through Diels-Alder reaction utilizing micro-contact printing of ligands. Three different polymers were synthesized with varying feed ratios of furfuryl methacrylate in order to tune the extent of immobilization on the reactive films. The reactive film containing highest amounts of furfuryl group was found to enable maximum enzyme immobilization (Figure 1.15).

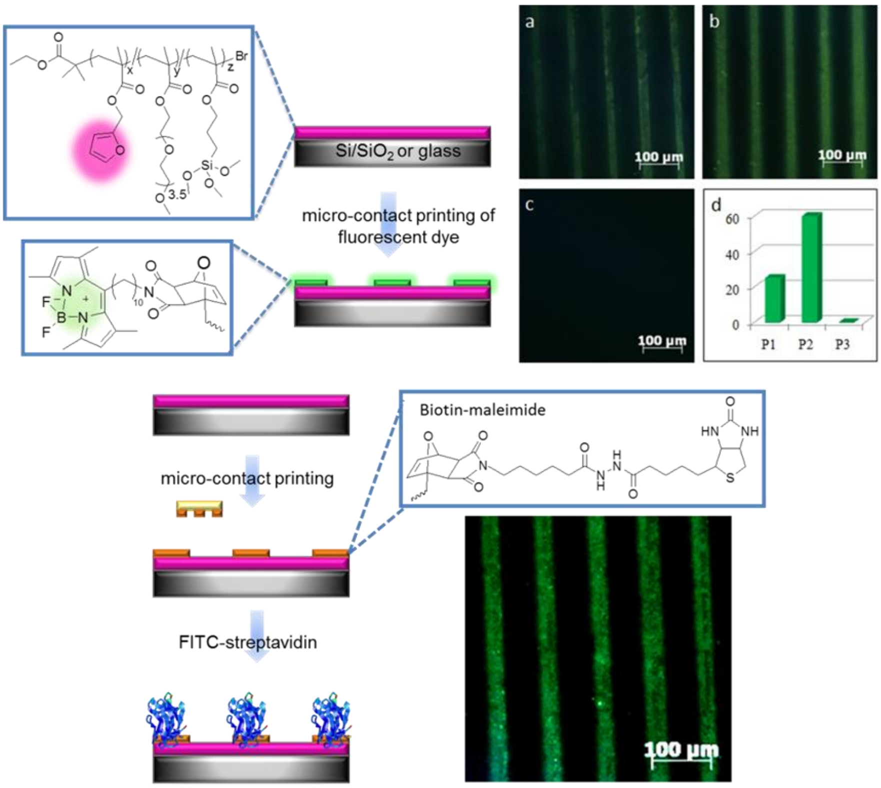


Figure 1.15. Functionalization of polymers coated surfaces via Diels-Alder reaction [39].

Moreover, in this research, the thermoreversibility of Diels-Alder reaction was demonstrated by reversible attachment of a fluorescent dye (Figure 1.16). Printed micro-patterns (“MCP”) on the polymeric surface through Diels-Alder were erased via the reverse reaction. After that, the re-functionalization was successfully demonstrated by re-writing new patterns using the Diels-Alder reaction.

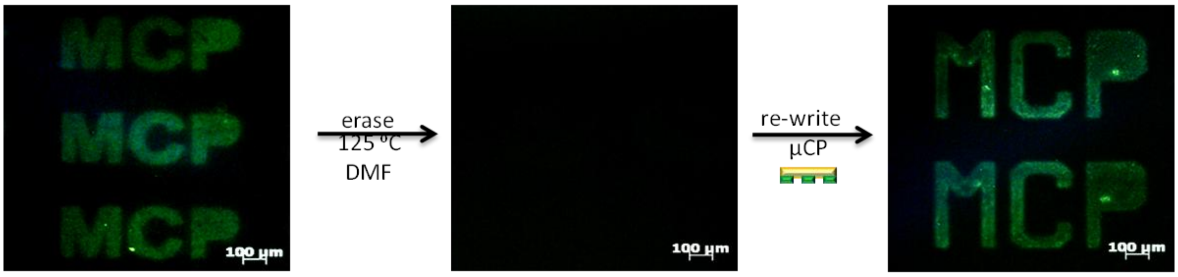


Figure 1.16. Reversible printing via Diels-Alder/retro-Diels-Alder reactions [39].

1.7. Polymer Surfaces Characterization

The following sections briefly describe the various characterization techniques used in this thesis and provide a reason for their choice.

1.7.1. Water Contact Angle

The water contact angle of a surface is a measure of hydrophilicity of this surface. A droplet of water is placed on the surface and the measured contact angle refers to the angle formed between this droplet and the surface. As seen in Figure 1.17, hydrophobic surfaces have high contact angle, whereas, hydrophilic surfaces have small contact angle. If a surface includes an abundance of ionizable groups or functional groups capable of doing hydrogen bonding with the water droplet, the droplet spreads on the surface and results in low contact angle [40]. Thus, water contact angle measurement can be used as a tool to understand changes in surface composition upon chemical modifications. In this thesis, contact angle measurements were used to observe the changement in the hydrophilicity of the surface befor and after initiator immobilization and after each of polymerization.

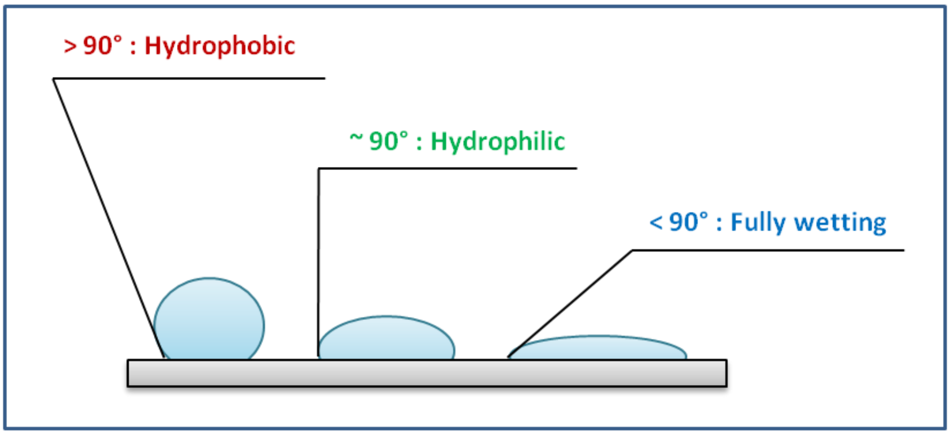


Figure 1.17. Contact angle measurement.

1.7.2. Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

ATR-FTIR allows the direct examination of a sample in the liquid or solid state in a few minutes without any special sample preparation. This method uses infrared (IR) radiation in order to obtain the spectrum of a surface and is used to identify chemical functional groups present on it. An IR beam passes through a diamond, ZnSe or Ge crystal, in contact with the analyzed surface. Chemical bonds of the surface absorb the applied IR radiation. After a total internal reflection, an evanescent wave is detected and enables to create the sample's absorbance spectrum (Figure 1.18). According to the functionality presents in the surface, absorption is taken at different wavenumber. Each chemical function absorbs at a known wavenumber, which make them easily recognizable. The spectrum obtained gives the absorbance or the transmittance varying with the wavenumber. The penetration depth of the IR beam depends on the crystal utilized in order to reflect the internal surface and the refractive index of the surface and is generally between 0.5 and 2.0 micrometers [41]. In this thesis, ATR-FTIR was used to characterize the surface before and after initiator immobilization, polymer brush growth and their functionalization.

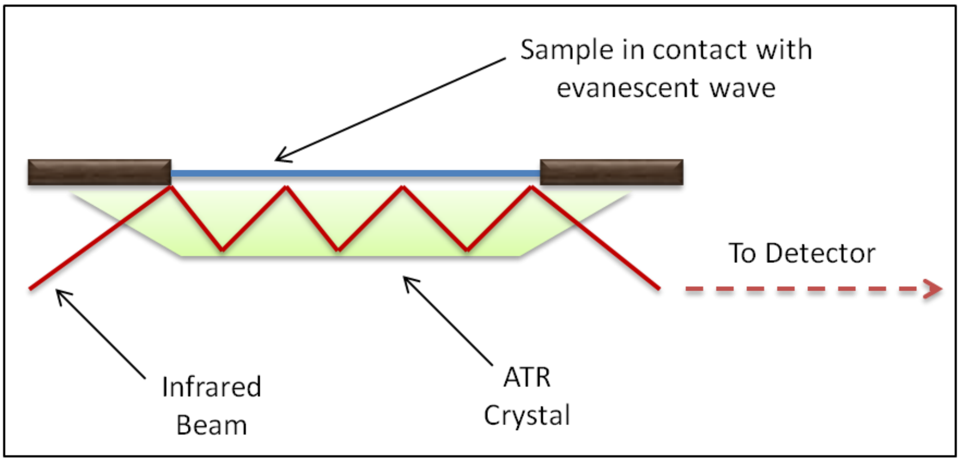


Figure 1.18. ATR-FTIR equipment.

1.7.3. Atomic Force Microscopy (AFM)

AFM is a method utilized in order to determine the morphology of a surface at the nanometer scale. The principle of this technique consists in the scanning of a surface by a flexible cantilever with a sharp tip at its end. A laser beam which comes from the cantilever is reflected in the direction of the detector (Figure 1.19). The difference in the surface thickness is determined by the position of this reflected laser beam. The roughness is calculated from a topographic image of the surface [42]. In this thesis, AFM was used to determine the polymer brush thickness varying with the duration of polymerization and the composition of the brush surface.

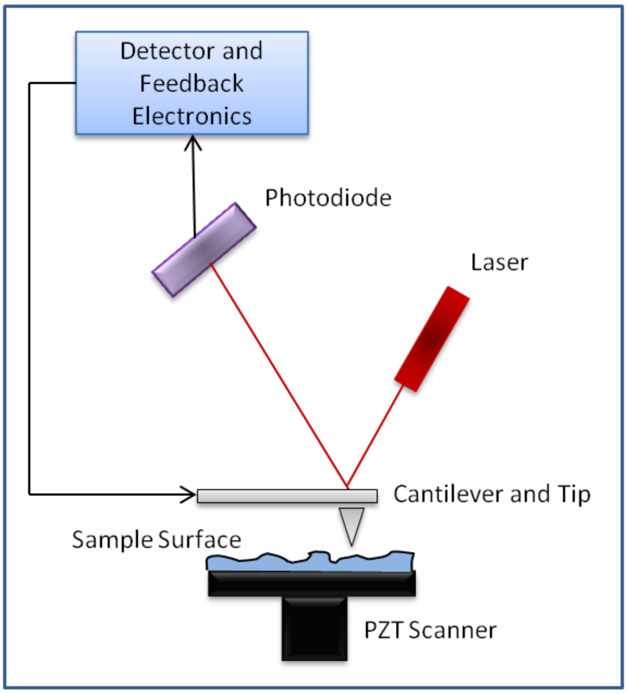


Figure 1.19. AFM equipment.

1.7.4. X-ray Photoelectrons Spectroscopy (XPS)

XPS is a spectroscopic method which allows the determination of atomic composition from the top about 10 nm deep of a surface. Principally, the surface is exposed to X-ray photons and emits photoelectrons with binding energies, which are comparable with known references [43]. The spectrum obtained gives the intensity varying with the binding energy (eV). The irradiated surface ejects photoelectrons with a certain intensity related to the atomic distribution of the surface (Figure 1.20). It is utilized in order to determine the stoechiometric ratios and atomic composition percentages of this surface [44]. Furthermore, by comparing the experimental atomic compositions with theoretical results, XPS enables to estimate the reaction extents. The theoretical values are obtained by assuming a total conversion of the reaction [45]. In this thesis, XPS was used to characterize the atomic composition of the polymer brushes before and after their functionalization.

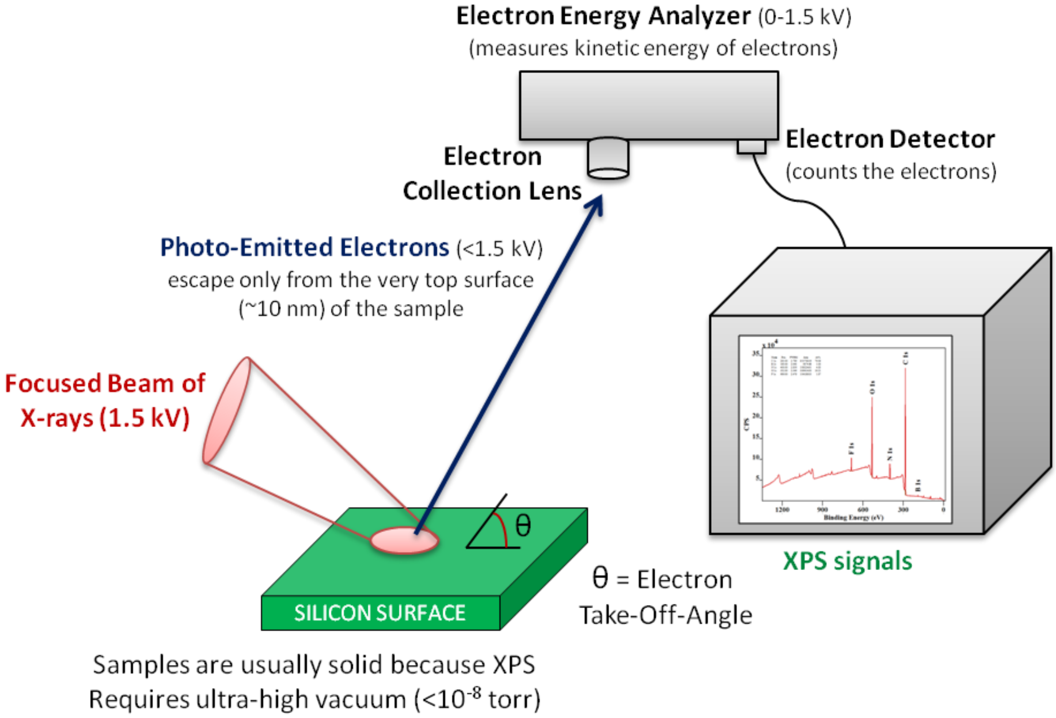


Figure 1.20. XPS equipment.

2. AIM OF THE STUDY

The aim of this study is the design of a novel reactive polymer brush coating that can be functionalized with molecules of interest without the use of catalysts. The reactive polymer brush will be synthesized via surface-initiated ATRP using the "grafting from" approach. Copolymers containing reactive furfuryl groups along with polyethylene glycol based side chains to provide antibiofouling properties will be synthesized. Furan groups present in polymer chain enable the conjugation of maleimidyl-compounds through Diels-Alder reaction. The retro-Diels-Alder reaction provides reversibility to this conjugation. Functionalization of polymer brushes will be studied using N-ethylmaleimide as a model compound. Finally, conjugation of a BODIPY-maleimide fluorescent dye and biotin-maleimide ligand immobilize streptavidin conjugates will be targeted.

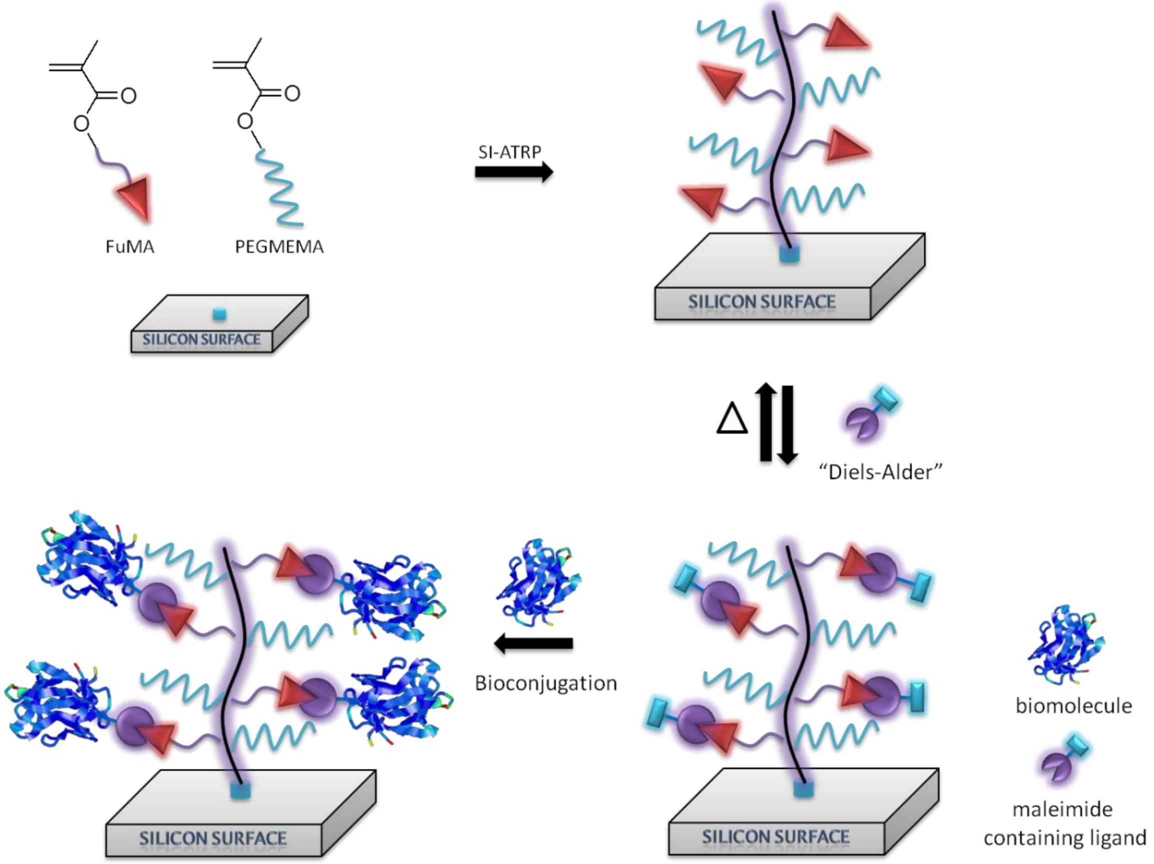


Figure 2.1. General scheme of the project.

3. RESULTS AND DISCUSSION

Polymer brush surfaces were synthesized by using surface-initiated atom transfer radical polymerization (SI-ATRP). The synthetic process consists of the modification of the silicon wafers with the initiators followed by the growth of polymer chains from these surfaces. First of all, modification of silicon surfaces with ATRP-initiator was realized by using an initiator containing a silylchloride group for surface attachment. Thereafter, poly(PEGMEMA-*ran*-FuMA) brushes were synthesized using SI-ATRP with varying monomers composition and the obtained brushes were analyzed by XPS, contact angle and FT-IR spectroscopy. Evolution of polymer brush thickness with polymerization time was also studied. Functionalization with a model dienophile, N-ethylmaleimide was studied. Furthermore, the thermoreversible aspect of Diels-Alder reaction was demonstrated using this model compound. Finally, the furan containing brushes were investigated for functionalization with a maleimide-containing fluorescent dye and a ligand for biomolecule immobilization (Figure 3.1).

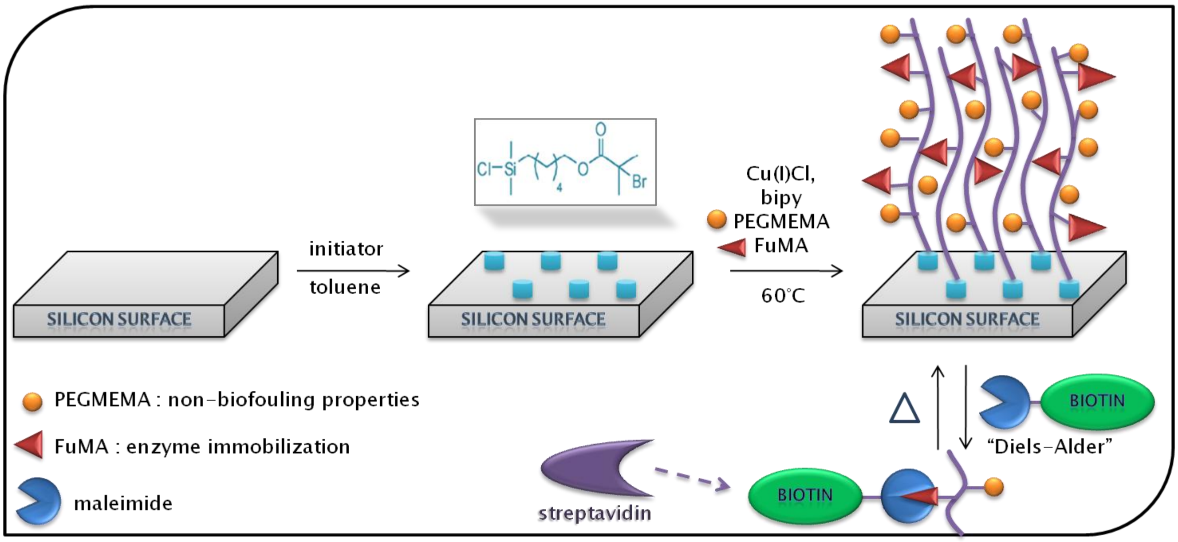


Figure 3.1. General scheme of the synthesis and functionalization of polymer brushes.

3.1. Modification of Silicon Surfaces with ATRP-initiator

The first step in the synthesis of polymer brushes involves the synthesis of the initiator, 6-(Chloro(dimethyl)silyl)hexyl 2-bromo-2-methylpropanoate. Then, this initiator was coated on silicon surfaces in order to realize the growth of polymer chains from these surfaces (Figure 3.2). The initiator was synthesized and coated on silicon surfaces according to previously reported literature procedure [46]. Briefly, after cleaning with detergent, water acetone and UV cleaner, respectively, Si/SiO_2 surfaces were dipped in a toluene solution containing the initiator (2 mM) for 16 hours to obtain a dense surface coating. The modified surfaces were rinsed with toluene and methanol and dried under a gentle stream of N_2.

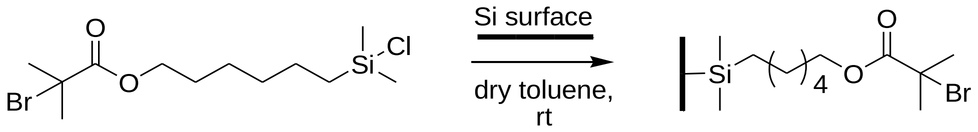


Figure 3.2. Modification of silicon surfaces with ATRP initiator.

The next step was to ascertain the efficiency of the initiator coating process. Contact angle measurements demonstrated that surface coated with the ATRP-initiator were hydrophobic, an increase in the contact angle from 27° to 82° was observed upon comparison with unmodified silicon wafer (Figure 3.3).

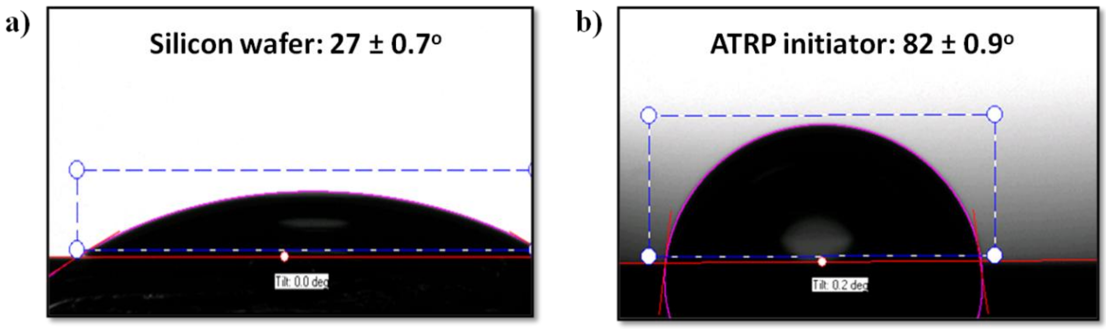


Figure 3.3. Contact angles values of clean (a) and initiator coated (b) silicon surfaces.

3.2. Polymerizations by SI-ATRP

Surface-initiated atom transfer radical polymerization (SI-ATRP) was used to synthesize the polymer brush surfaces since SI-ATRP is a living polymerization technique that gives precise control over polymer brush thickness and composition.

Polymer brushes synthesized in this research utilize furfuryl methacrylate (FuMA) and poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) as monomers. As mentioned previously, FuMA was used in order to obtain reactive polymeric surfaces. The hydrophilic monomer PEGMEMA was utilized to tender hydrophilic and antibiofouling properties to the surfaces. Choice of these monomers was defined by the aim to obtain specific conjugation of molecules of interest while reducing the unwanted non-specific adhesion, hence provide a higher signal-noise ratio during detection.

The polymerization starts from the alkyl halide part of the initiator with the addition of the monomers units in the presence of a metal catalyst. A Cu(I)Cl/2,2-bipyridine complex was used as a catalyst in this study, since this has been shown to be an effective system in previous studies for growth of hydrophilic polymer brushes. The polymerization was carried out in aqueous environment using a mixture of methanol and water in order to solubilize two monomers (Figure 3.4).

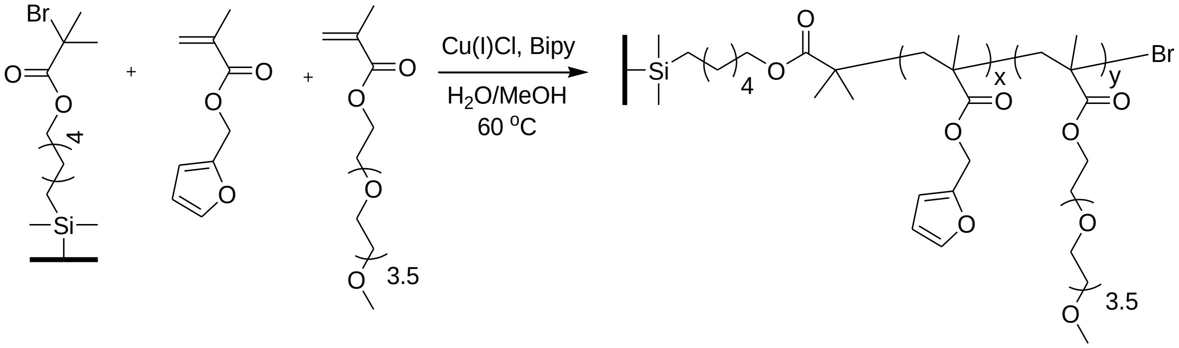


Figure 3.4. Polymer brushes synthesis via SI-ATRP.

In order to probe the tunability of extent of functionalization, several polymers containing different ratios of FuMA and PEGMEMA (90/10 PEGMEMA/FuMA, 75/25 PEGMEMA/FuMA and 60/40 PEGMEMA/FuMA) were synthesized. Brushes with varying thickness could be obtained by controlling the duration of polymerization. While the PEGMEMA monomer is hydrophilic, the FuMA monomer is hydrophobic. Hence, a change in water contact angle of the brush surface can be expected for different compositions. Figure 3.5 shows the increasing hydrophobicity of the surface when the amount of FuMA was increased in the polymer. The contact angle was 68º, 72º and 79º for 90/10, 75/25, 60/40 PEGMEMA/FuMA, respectively.

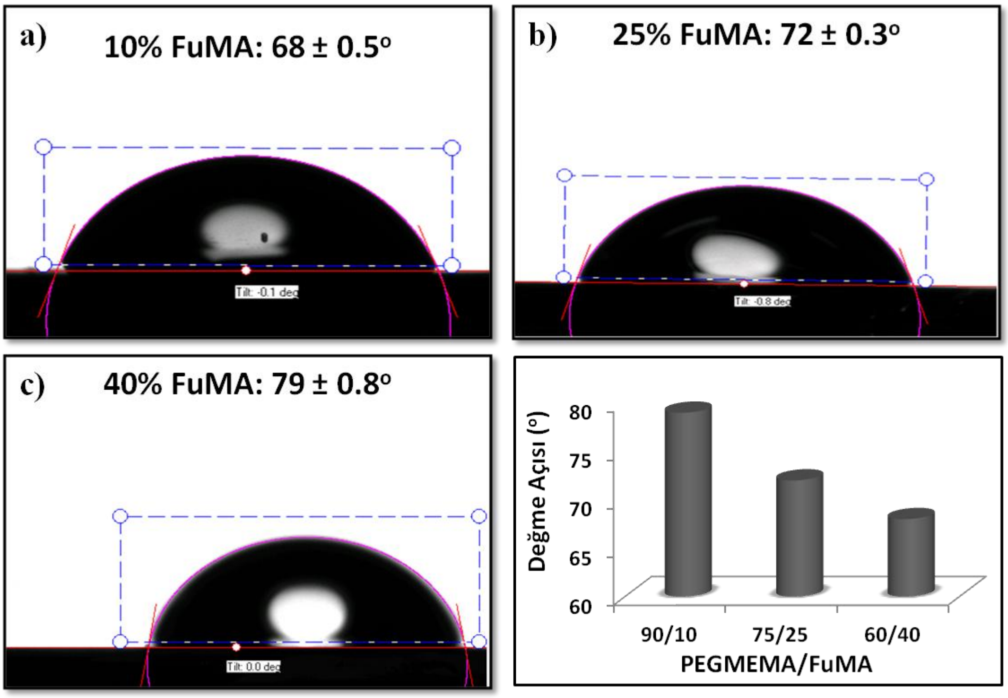


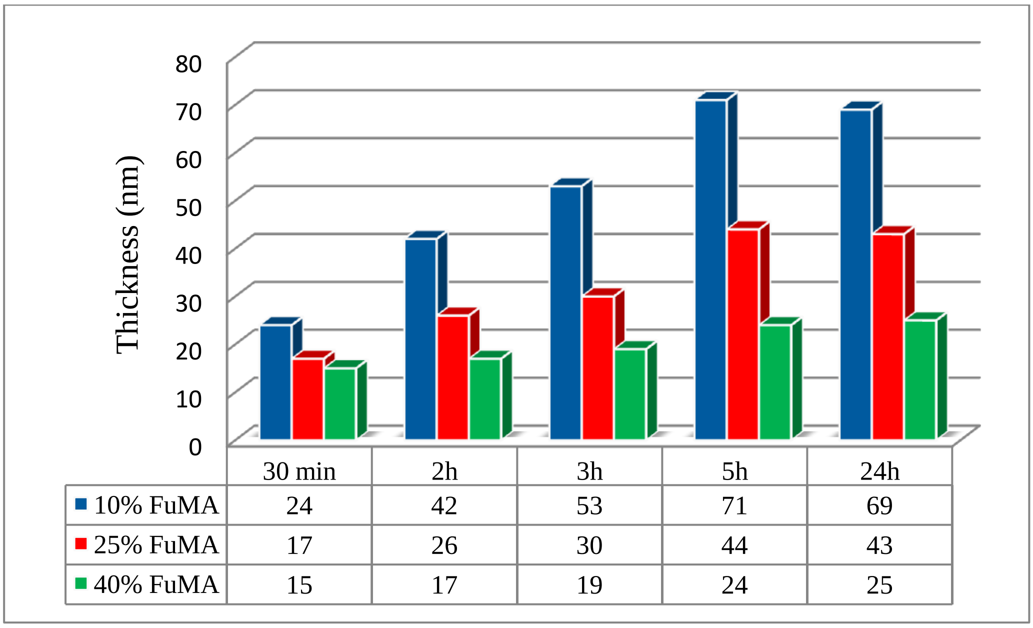
Figure 3.5. Contact angle of (a) 90/10 PEGMEMA/FuMA, (b) 75/25 PEGMEMA/FuMA, (c) 60/40 PEGMEMA/FuMA polymer brush surfaces.

3.3. Evolution of Brush Thickness with Polymerization Time

The evolution of brush thickness with polymerization time was evaluated. In order to realize this, three sets of polymers containing different ratios of PEGMEMA/FuMA were prepared. In each set, five polymers were synthesized with different polymerization time. Thickness of the polymer films were determined by atomic force microscopy (AFM).

The analysis of the evolution of polymer brush thickness with polymerization time (Table 3.1), shows that in one hand, the thickness decreases when the amount of the reactive group FuMA increases in the polymer brushes. In the other hand, for the same group of polymers having identical monomers composition, it exhibits an increase in the polymer films until a certain time (5h) and after this time the thickness reaches a plateau.

Table 3.1. Thickness evolution of polymer brush films with polymerization time for 90/10 PEGMEMA/FuMA (blue), 75/25 PEGMEMA/FuMA (red), 60/40 PEGMEMA/FuMA (green) polymer brushes.



	30 min	2h	3h	5h	24h
🟦 10% FuMA	24	42	53	71	69
🟥 25% FuMA	17	26	30	44	43
🟩 40% FuMA	15	17	19	24	25

3.4. Functionalization of Polymer Brushes

Polymer brush surfaces were functionalized with N-ethylmaleimide, which was chosen as a model compound to analyze the efficient reversible functionalization of the polymer brushes. BODIPY-maleimide was then immobilized on polymer brush films to tunable the observations by fluorescence microscopy. Finally, the immobilization of the biomolecule streptavidin was investigated by using a biotin-maleimide ligand and Quantum-Dots streptavidin conjugates (Figure 3.6).

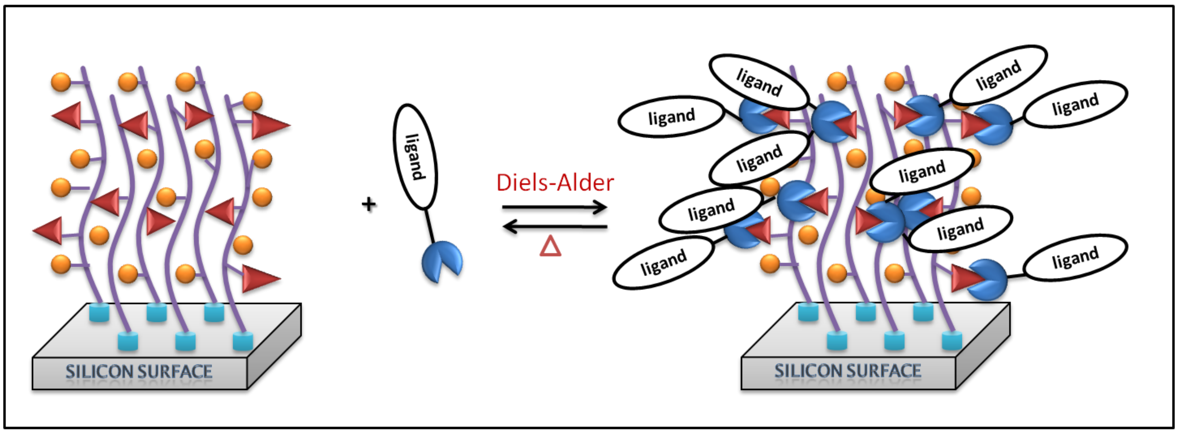


Figure 3.6. Thermoreversible functionalization of polymer brush surfaces.

3.4.1. Model studies to probe the functionalization of polymer brushes N-ethylmaleimide

3.4.1.1. Functionalization and Analysis of N-ethylmaleimide functionalized brushes.

Diels-Alder reaction occurs easily at room temperature without addition of any additional chemical reagents. This reaction takes place between a diene and a dienophile. In this research study, furan group is used as the diene component and maleimide group is chosen as a dienophile. Additionally, obtained cycloadduct can undergo retro Diels-Alder reaction when heated above a certain temperature.

N-ethylmaleimide was chosen as a model compound to study the functionalization of side chain furan containing polymer brushes through Diels-Alder cycloaddition reaction due to its similarity to substituted maleimides that would be used as functional molecules (Figure 3.7). Degree of functionalization studies of the polymers containing different ratios of FuMA was performed by using this compound, and characterized with X-Ray Photoelectron Spectroscopy (XPS) and Infrared Spectroscopy (IR).

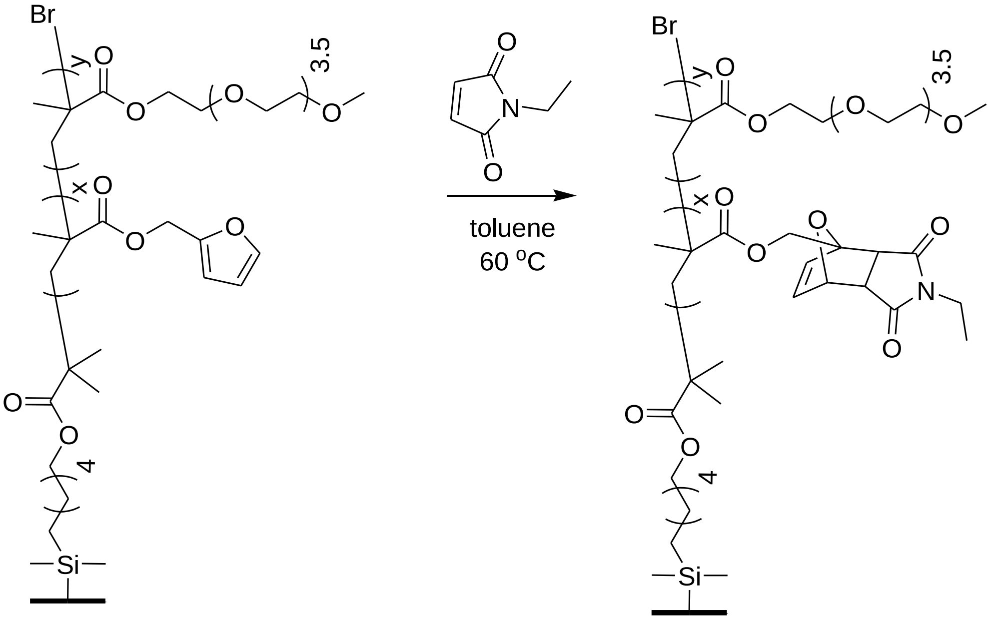
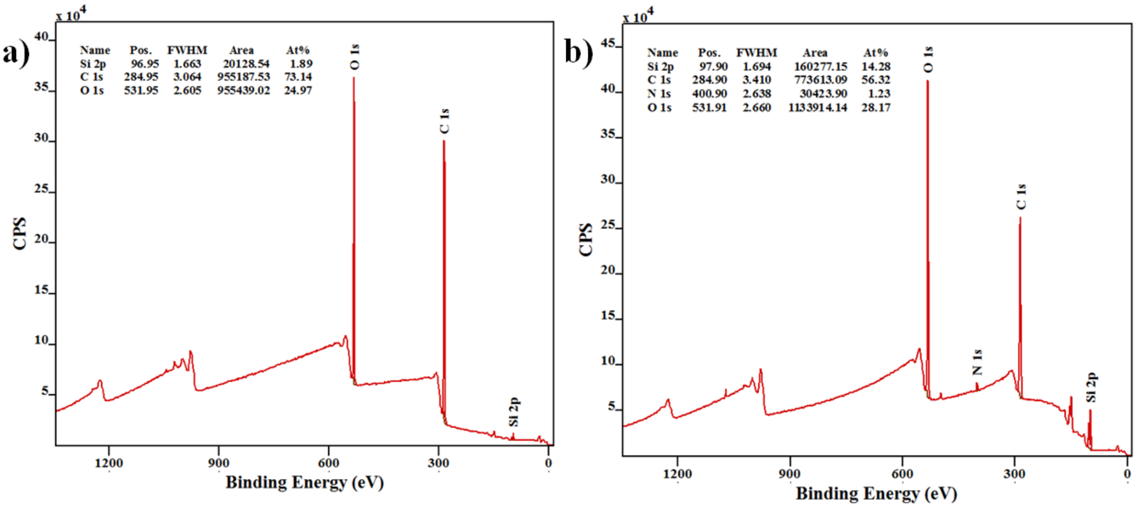


Figure 3.7. Functionalization of polymer brushes with N-ethylmaleimide through Diels-Alder reaction.

Additionally, N-ethylmaleimide contains a nitrogen atom that is not present in the original polymer backbone, hence provides a useful marker for the analysis of the functionalization with XPS measurements. Comparing the surveys before (Figure 3.8a) and after (Figure 3.8b) functionalization with N-ethylmaleimide reveals the occurance of functionalization. The N (1s) peak (at 400 eV) corresponding to the nitrogen atom appears for the survey scan after functionalization with N-ethylmaleimide.



Name	Pos.	FWHM	Area	At%
Si 2p	96.95	1.663	20128.54	1.89
C 1s	284.95	3.064	955187.53	73.14
O 1s	531.95	2.605	955439.02	24.97

Name	Pos.	FWHM	Area	At%
Si 2p	97.90	1.694	160277.15	14.28
C 1s	284.90	3.410	773613.09	56.32
N 1s	400.90	2.638	30423.90	1.23
O 1s	531.91	2.660	1133914.14	28.17

Figure 3.8. (a) XPS survey of poly(PEGMEMA-*ran*-FuMA brush. (b) XPS survey of N-ethylmaleimide functionalized poly(PEGMEMA-*ran*-FuMA) brush.

3.4.1.2. Comparison of N-Ethylmaleimide functionalized Polymer Brushes containing different ratios of FuMA. A series of polymers were synthesized in order to tailor their functionalization. The polymers were synthesized with varying FuMA/PEGMEMA composition. After functionalization with N-ethylmaleimide, the resulting polymer films were analyzed with X-Ray Photoelectron Spectroscopy (XPS). In Figure 3.9, the XPS spectra of the nitrogen atoms of each polymer are shown, according to these spectra increasing furan groups results in increased amount of nitrogen content. Hence, it is clearly seen that functionalization degree of reactive polymer films can be controlled by increasing feed ratio of furfuryl methacrylate. Figure 3.10 tabulates both theoretical and experimental percentages and percentages of nitrogen atom increases with the increasing ratios of furfuryl methacrylate in the polymer brushes. From comparison of experimental (Figure 3.10a) and theoretical (Figure 3.10b) percentages of nitrogen atom, the experimental ones seem to be half of the theoretical ones (Figure 3.10c). This suggests that about half of the available furan groups participate in the Diels-Alder reaction with N-ethylmaleimide. This difference is due to the steric hinderance formed between the polymer chains of the polymer brush. This steric hinderance establishes a barrier for the incoming N-ethylmaleimide molecules. One can expect that dilution of the grafting density of the polymer chains will provide improved accessibility to the reactive group, thus improving overall extent of functionalization.

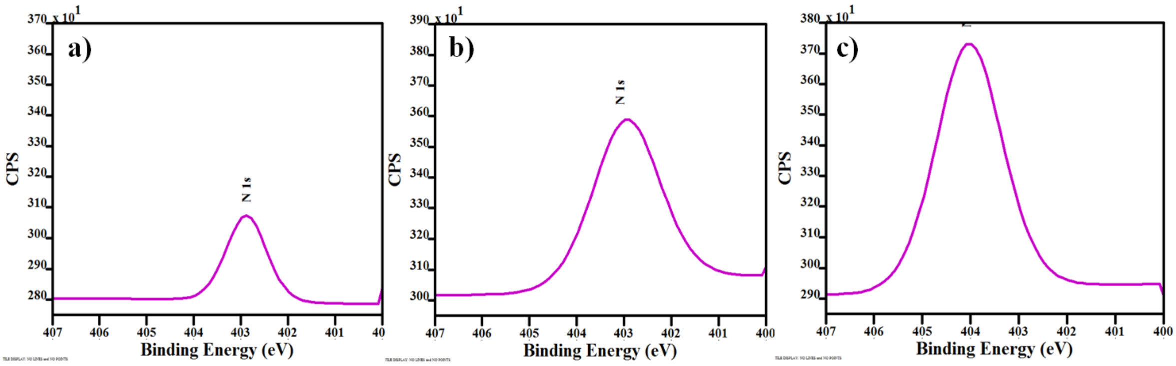


Figure 3.9. High resolution XPS elemental scan of N1s peak of N-ethylmaleimide functionalized poly(PEGMEMA-*ran*-FuMA) brushes composed by (a) 90/10, (b) 75/25, (c) 60/40 PEGMEMA/FuMA.

a)

Experimental	10% FuMA	25% FuMA	40% FuMA
C 1s	74%	75.41%	74.58%
O 1s	25.76%	24.03%	24.49%
N 1s	0.24%	0.55%	0.93%

b)

Theoretical	10% FuMA	25% FuMA	40% FuMA
C 1s	68.6%	69%	69.6%
O 1s	30.9%	29.7%	28.5%
N 1s	0.49%	1.21%	1.93%

c)

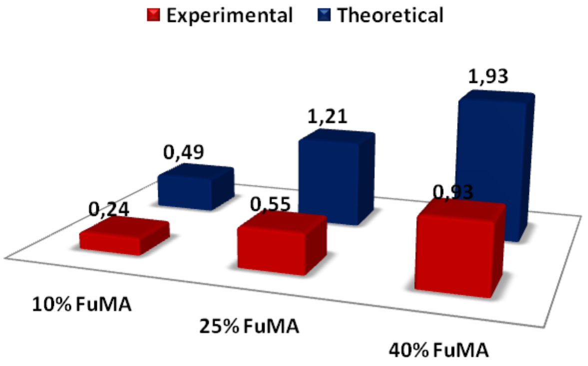


Figure 3.10. (a) Experimental and (b) theoretical percentages of the 10%, 25% and 40% FuMA containing brushes in C 1s, O 1s and N 1s compositions obtained by XPS. (c) XPS results of Nitrogen atom% in N-ethylmaleimide functionalized poly(PEGMEMA-*ran*-FuMA) brushes with 10%, 25% and 40% FuMA compositions.

Further characterization of the Diels-Alder reaction was obtained from the ATR-IR analysis of the polymer brushes. The attachment of N-ethylmaleimide to furan molecules of the polymer brushes was clear, because the carbonyl (C=O) strech of the homopolymer poly(PEGMEMA) peak around 1701 cm^{-1}, an additional peak appeared at around 1730 cm^{-1}. This additional peak comes from the carbonyl group on the maleimide unit. As expected, when the amount of furan groups increased in the polymer brushes, an increase in the stretching of the imide carbonyl bond was observed (Figure 3.11).

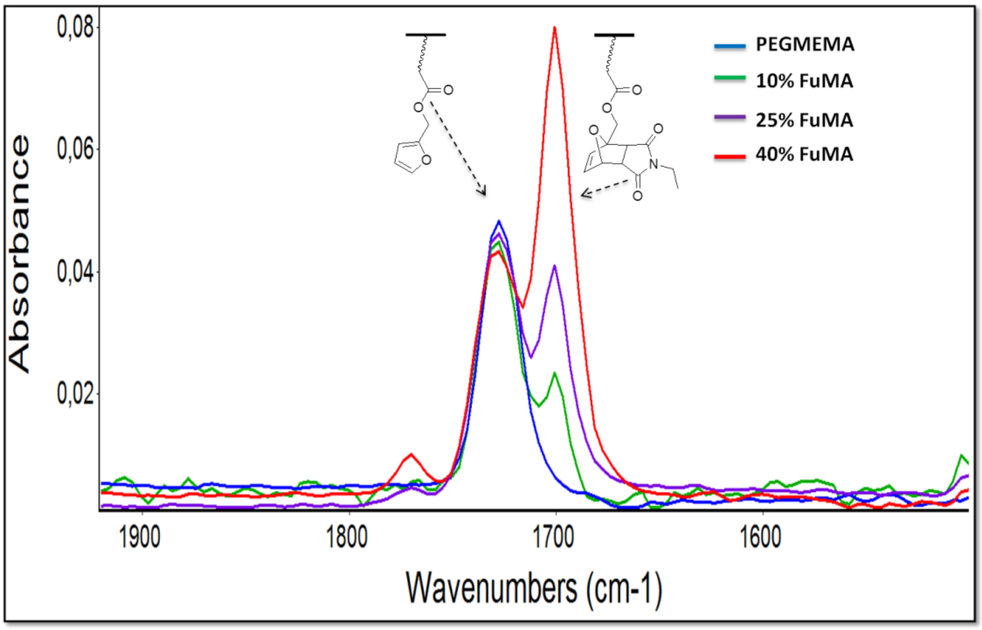


Figure 3.11. ATR-FTIR spectra of carbonyl regions for poly(PEGMEMA) and for poly(PEGMEMA-*ran*-FuMA) brushes with 10%, 25% and 40% FuMA compositions.

<u>3.4.1.3. Diels-Alder reaction and retro-Diels-Alder</u>. Diels-Alder reaction was used to functionalize polymer brushes with N-ethylmaleimide. Indeed, when the temperature was increased up to 110 ºC the reverse reaction occurs without additional reagents (Figure 3.12).

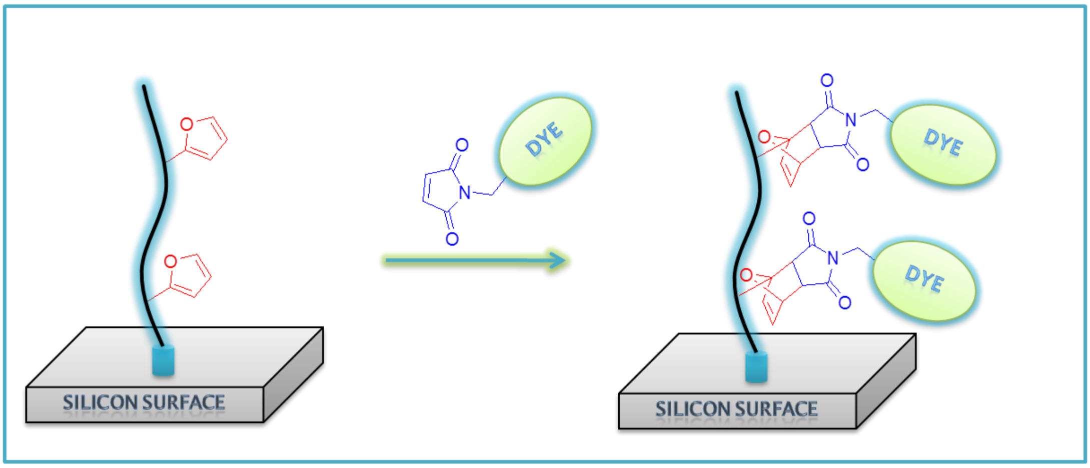


Figure 3.12. Diels-Alder reaction and retro-Diels-Alder.

In this research, after functionalization of polymer brush surfaces with N-ethylmaleimide at 60 ºC, the surfaces were exposed to higher temperatures (110 ºC). The retro-Diels-Alder reaction takes place and the polymer brush surfaces revert back to their original statues (Figure 3.13a). The renewed polymeric surfaces can be functionalized again with N-ethylmaleimide through the Diels-Alder reaction (Figure 3.13b).

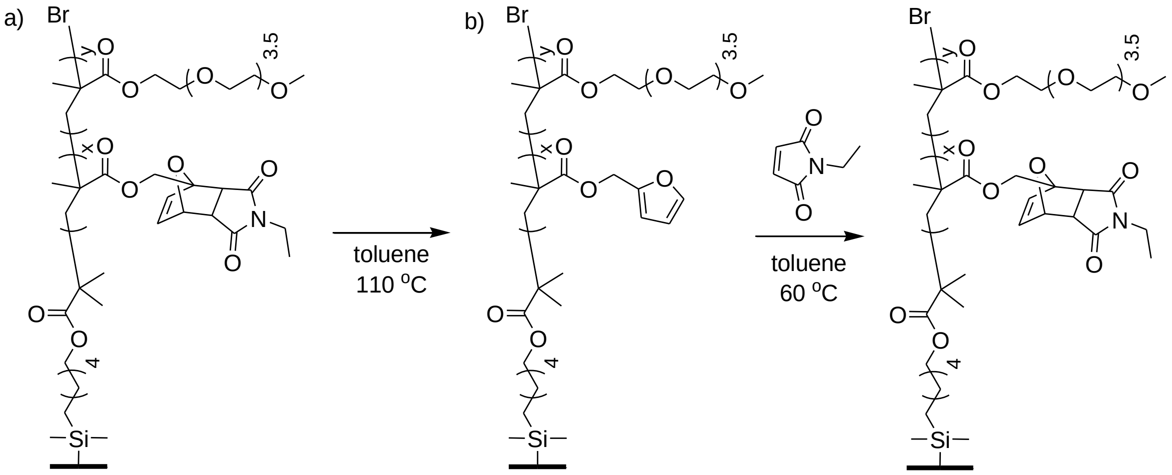


Figure 3.13. Retro-Diels-Alder reaction at the first step and Diels-Alder reaction at the second step.

Extent of functionalization and the reverse process was followed by XPS.

XPS elemental scan of N1s peak, showing after attachment of N-ethylmaleimide on polymer brushes (Figure 3.14a), disappears with retro-Diels-Alder reaction (Figure 3.14b). This peak reappears after N-ethylmaleimide attachment through Diels-Alder reaction (Figure 3.14c).

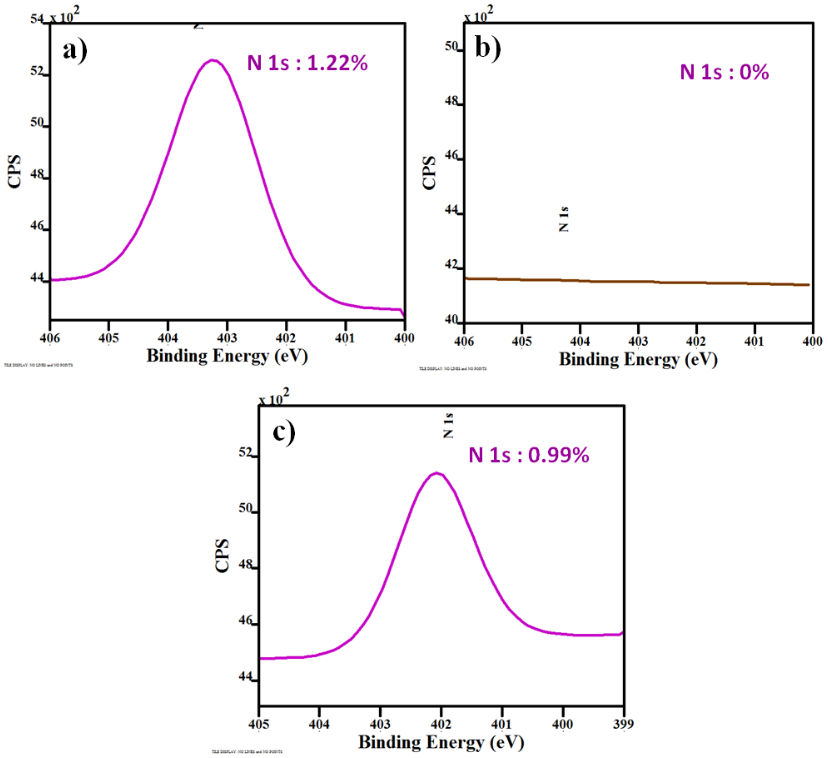


Figure 3.14. High resolution XPS elemental scan of N1s peak of N-ethylmaleimide functionalized poly(PEGMEMA-*ran*-FuMA) brushes (a) after Diels-Alder reaction; (b) after retro-Diels-Alder reaction; (c) after Diels-Alder reaction again.

ATR-IR spectra of the polymer brushes also prove the succesful functionalization with maleimide and its erasing from surface via Diels-Alder and retro Diels-Alder reactions. In the first spectra, the carbonyl (C=O) groups stretch at around 1701 cm^{-1} with a supplementary stretching at around 1730 cm^{-1} are seen. These two peaks belong to metacrylate and maleimide carbonyls, respectively (Figure 3.15a). After the retro Diels-Alder reaction, carbonyl stretch which corresponds to maleimide group disappears (Figure 3.15b). After functionalization with N-ethylmaleimide, the carbonyl stretch belonging to the maleimide unit reappars at 1730 cm^{-1} (Figure 3.15c).

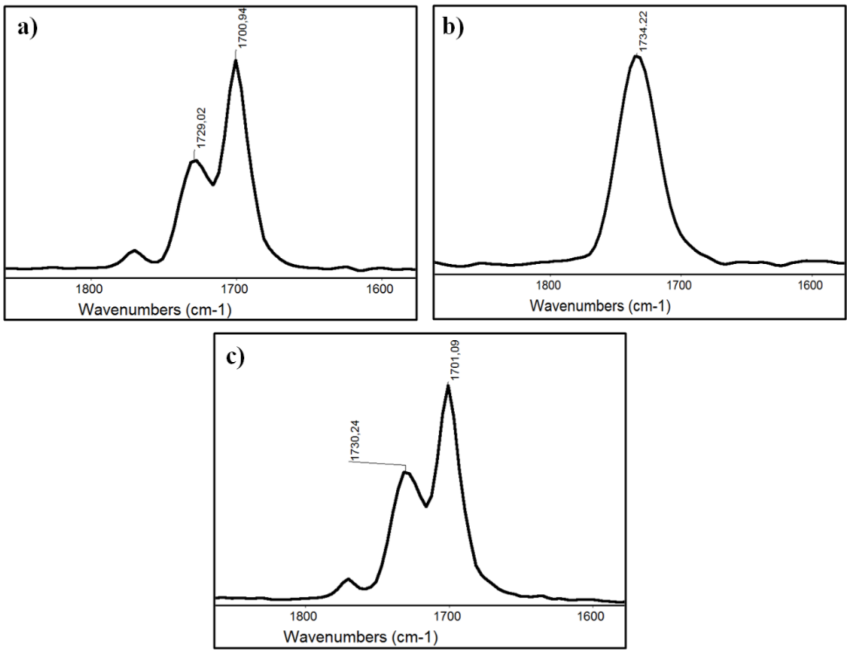


Figure 3.15. ATR-FTIR spectra of carbonyl regions of poly(PEGMEMA-ran-FuMA) brushes (a) after Diels-Alder reaction; (b) after retro-Diels-Alder reaction; (c) after Diels-Alder reaction again.

3.4.2. Immobilization of BODIPY-maleimide

BODIPY is a fluorescent dye molecule that was introduced by Treibs and Kreuzer in 1968. This compound has many advantageous such as its solubility in almost all of organic solvents, its high fluorescence and high photostability [47].

In this research, BODIPY was used to demonstrate the functionalization of the synthesized polymer brushes using fluorescence microscopy. All synthetic steps to obtain this dye are illustrated in Figure 3.16. Briefly, BODIPY-N_3 was conjugated to a furan-protected alkyne-containing maleimide group. After the conjugation, retro Diels-Alder reaction was used to unmask the maleimide group on the dye. Chemical composition of purified dye was established using ^{1}H NMR, and CHNS analysis. The ^{1}H NMR spectrum shows the characteristic peaks of this compound at around 6 ppm, 2.4 ppm and 2.5 (Figure 3.17). In addition to these peaks, as BODIPY-maleimide was synthesized with “Click” reaction in this method, there is the characteristic peak which comes from the “triazole” at around 7.5 ppm.

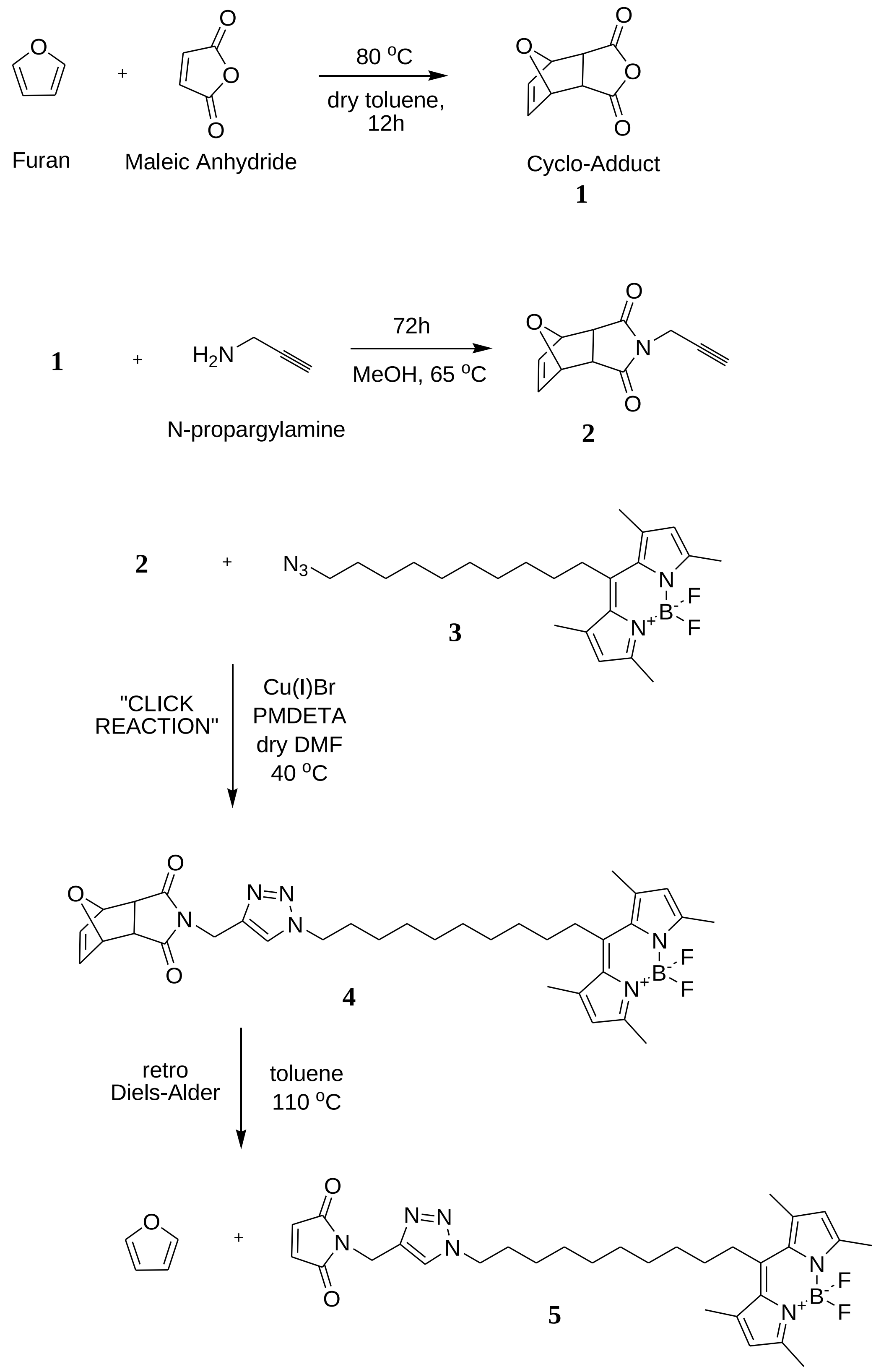


Figure 3.16. Synthesis of BODIPY-maleimide.

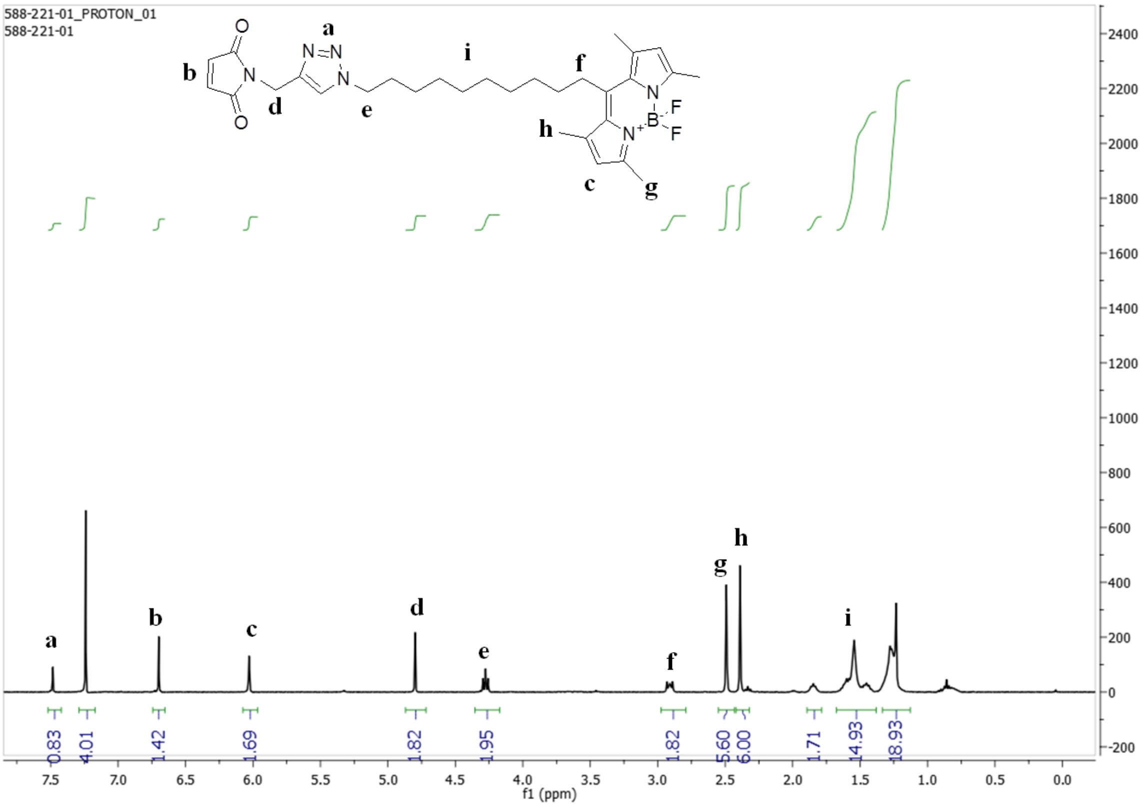


Figure 3.17. ^{1}H NMR spectrum of BODIPY-maleimide.

The experimental results of CHNS elemental analysis C: 62.36%, N: 10.43%, H: 8.45% are in agreement with the theoretical ones C: 63.83%, N: 14.89%, H: 6.96%.

The fluorescent dye molecule, BODIPY-maleimide, was attached to polymer films with Diels-Alder reaction and erasing from the surface via retro Diels-Alder reaction (Figure 3.18). Functionalization was accomplished by incubation of the polymer brush coated surface in a solution of dye at 60 °C. De-conjugation of dye molecules was achieved by heating the functionalized surfaces in a solution of toluene at 110 °C. The process of attachment and detachment was followed by fluorescent microscopy. As seen in Figure 3.19a, patterned polymer brush functionalized by BODIPY-maleimide were highly fluorescent. After retro Diels-Alder reaction, fluorescence was substantially diminished (Figure 3.19b). To prove polymer brush patterns can be reusable for second functionalization, the same surface was functionalized with the dye molecule again. Refunctionalization was demonstrated successfully using fluorescence microscopy (Figure 3.19c).

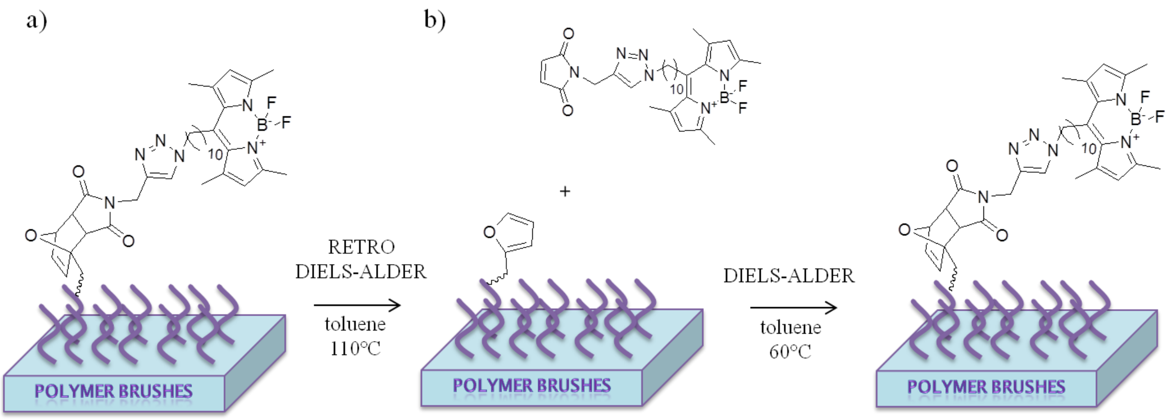


Figure 3.18. (a) Retro-Diels-Alder reaction of BODIPY-maleimide; (b) Diels-Alder reaction for the immobilization of BODIPY-maleimide.

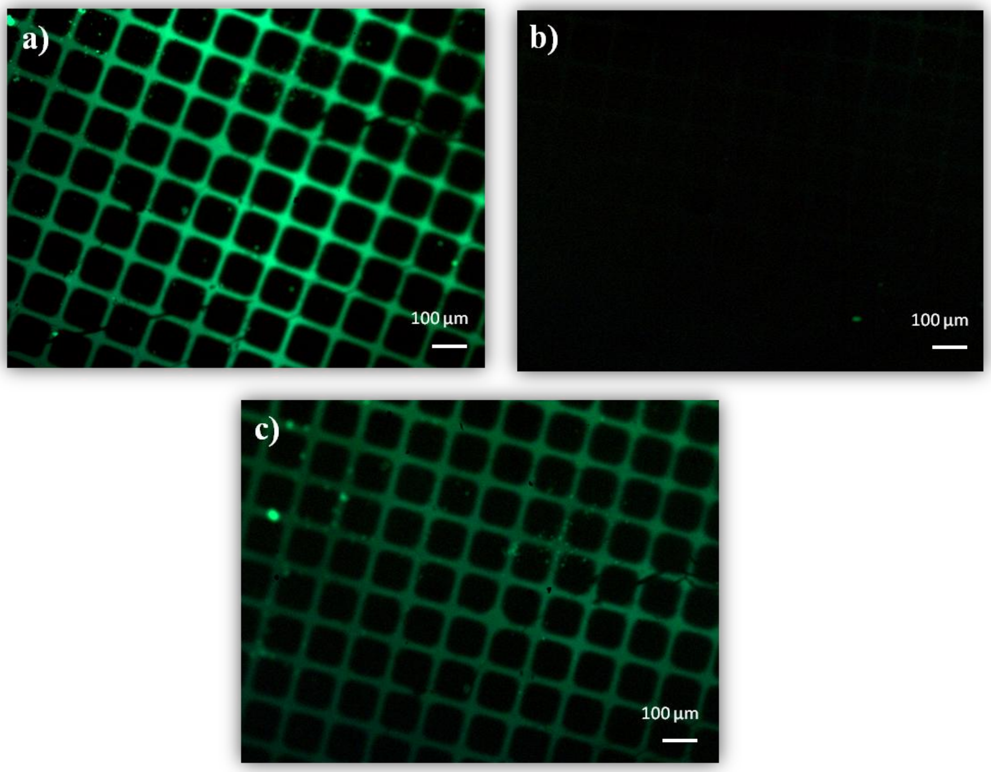


Figure 3.19. Fluorescence images of polymer brushes functionalized with BODIPY-maleimide, (a) after Diels-Alder; (b) after retro-Diels-Alder; (c) after second Diels-Alder reaction.

The XPS analysis of BODIPY-maleimide attached brushes, shows the F (1s), N (1s) and B (1s) peaks, which prove the effective binding of BODIPY-maleimide on the polymer brush (Figure 3.20a). After the retro Diels-Alder reaction, F (1s) and B (1s) peaks are disappeared as expected and N (1s) peak is almost disappeared (Figure 3.20b). This demonstrates the succesful detachment of BODIPY-maleimide from the polymer surface.

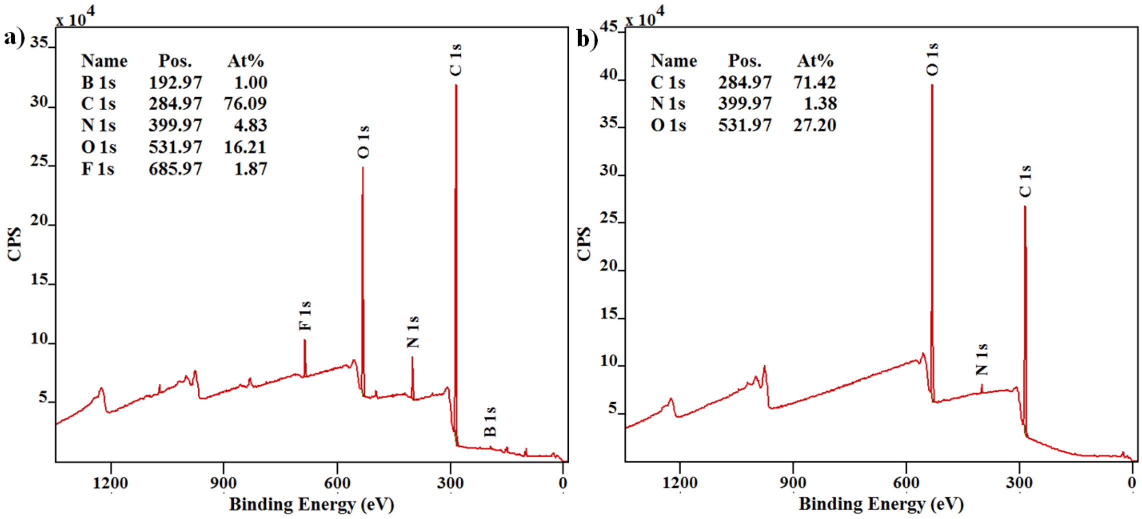


Figure 3.20. XPS survey of BODIPY-maleimide functionalized poly(PEGMEMA-*ran*-FuMA brush, (a) before retro-Diels-Alder and (b) after retro-Diels-Alder.

3.4.3. Immobilization of Biomolecules

A ligand-mediated immobilization of protein was targeted. Biotin is a ligand that is known to possess a high affinity for the protein streptavidin. So we aimed for functionalization of polymeric brushes with biotin-maleimide. These biotinylated polymeric brushes can be functionalized using streptavidin or streptavidin conjugates (Figure 3.21).

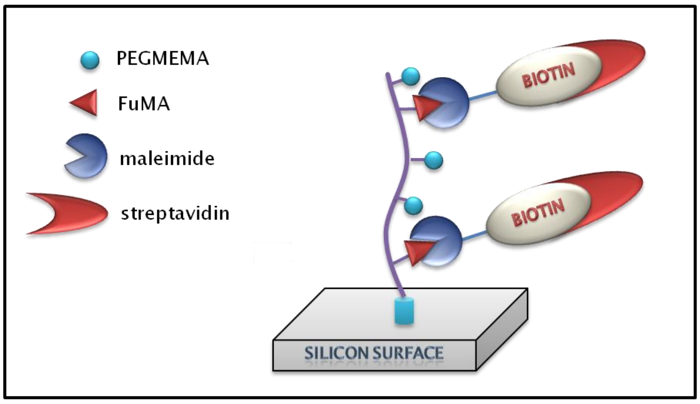


Figure 3.21. Immobilization of biomolecules on polymer brushes.

3.4.3.1. Functionalization with Biotin-maleimide. After successful functionalization with a fluorescence dye molecule was established, biomolecule immobilization studies were performed. Biotin-maleimide was attached to pendant furfuryl bearing polymer brushes in DMSO at 60 °C (Figure 3.22). Modification of polymeric brushes with biotin-maleimide was followed by XPS spectrum. Based on comparison of theoretical and experimental atomic compositions, a 50% functionalization was realized (Figure 3.22). The XPS analysis demonstrates the binding of this molecule on polymeric surface with the presence of nitrogen and sulfur peaks (Figure 3.23).

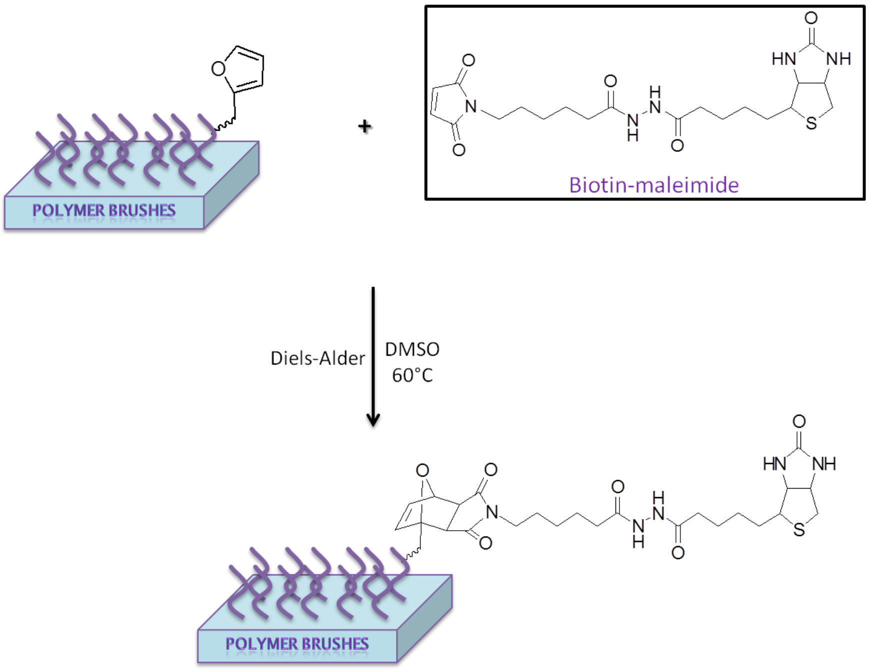
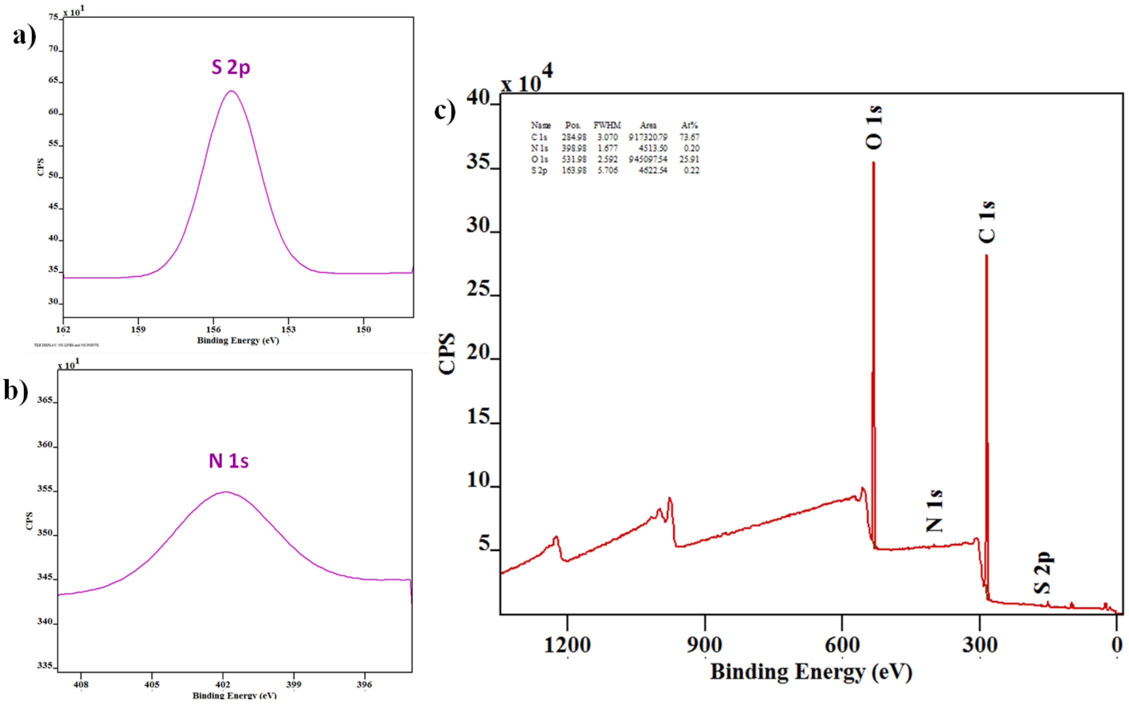


Figure 3.22. Immobilization of Biotin-maleimide through Diels-Alder reaction.



Name	Pos.	FWHM	Area	At%
C 1s	284.98	3.070	917320.79	73.67
N 1s	398.98	1.677	4513.50	0.20
O 1s	531.98	2.592	945097.54	25.91
S 2p	163.98	5.706	4622.54	0.22

Figure 3.23. (a) S (2p) peak, (b) N (1s) peak and (c) XPS survey of biotinylated polymer brushes.

3.4.3.2. Immobilization of streptavidin conjugate Q-Dots to biotinylated brushes. In the second step, streptavidin conjugated quantum dots (Q-Dots) were attached to biotinylated polymeric surfaces (Figure 3.24). This immobilization was verified by fluorescence microscopy (Figure 3.25a). As a control, incubation of non-biotinylated polymer brushes with a solution of streptavidin conjugated Q-dots, minimal fluorescence was observed (Figure 3.25b). This control experiment also shows that streptavidin conjugated quantum dots did not adsorbed non-specifically as expected due to presence of the anti-biofouling PEG-based chains.

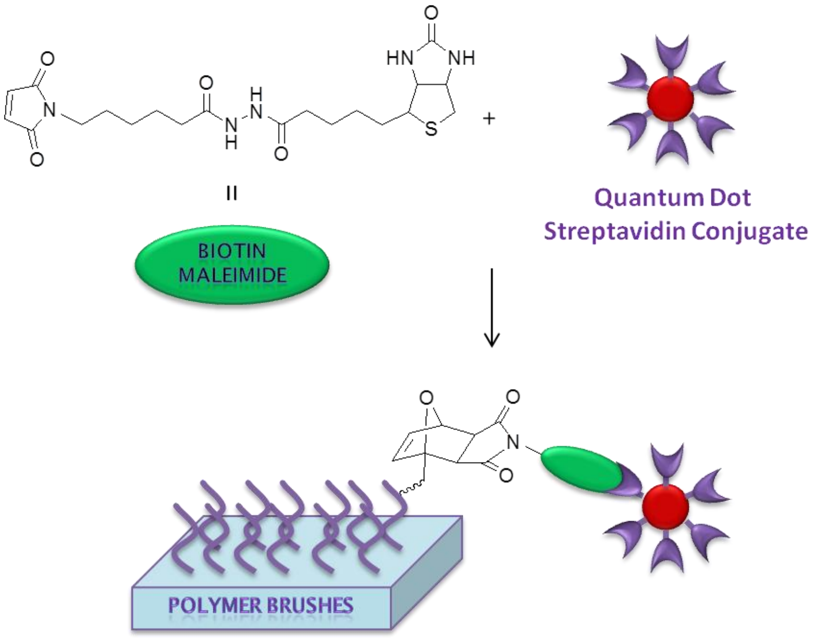


Figure 3.24. Immobilization of streptavidin conjugated Q-Dots on biotinylated polymer brush surface.

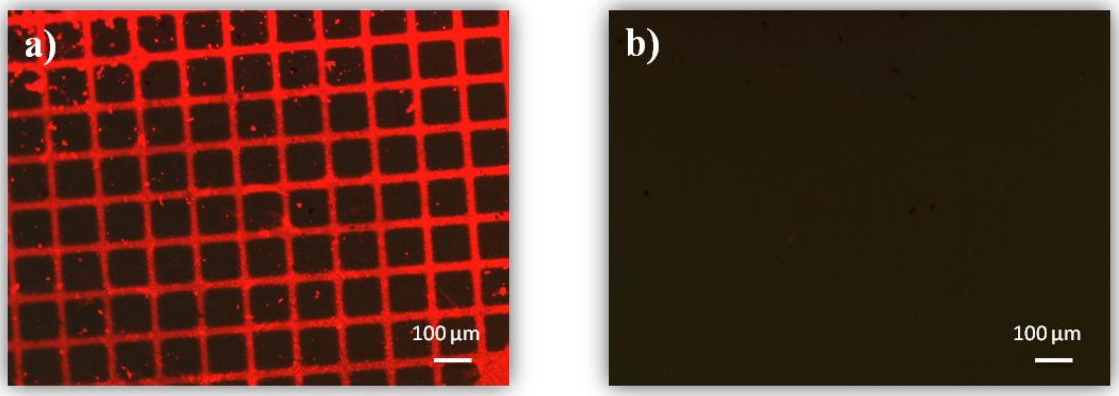


Figure 3.25. Fluorescence microscopy images of streptavidin conjugated Q-dots attached, (a) patterns and (b) control.

4. EXPERIMENTAL

4.1. Materials

5-hexen-1-ol (98%), 2-bromo-2-methylpropanoyl bromide (98%), chlorodimethylsilane, platinum on carbon (10 wt.% loading), furfuryl methacrylate (FuMA, 97%), polyethylene glycol methyl ether methacrylate (PEGMEMA, Mn: 300 g/mol), 2,2'-Bipyridine and biotin-maleimide (≥ 95%) were purchassed from Sigma-Aldrich. Cu(I)Cl (99%) was obtained from Acros Organics. The monomers FuMA and PEGMEMA were passed through aluminium oxide to remove the inhibitors and become activated. Organic solvents were obtained from Merck. Milli-Q water (18.2 MΩ cm) was taken from a Millipore Direct-Q 5 ultrapure water system. Qdot® 605 streptavidin conjugate was purchassed from Invitrogen molecular probes.

4.2. General Methods

Prior to initiator immobilization, substrates were cleaned using a Novascan *PSD Series UV/Digital Ozone System* for 15 minutes. Static water contact angles were determined using a KSV's CAM 101 on the non-patterned part of a polymer brush coated substrate. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was performed on a Thermo Scientific Nicolet 380 FT-IR spectrophotometer equipped with Harrick Scientific GATR accessory and a Ge crystal. An initiated silicon wafer was used as a background. The measurements were performed on non-patterned regions of polymer brush films. Atomic force microscopy was performed on an Ambios-Quesant Q-Scope Universal SPM (Scanning Probe Microscope). To determine layer thicknesses, cross-sectional height profiles of patterned polymer brushes were recorded. X-ray photoelectron spectroscopy (XPS) was realized on the non-patterned regions of a polymer brush by means of a K-Alpha instrument from Thermo Scientific. The X-ray source employed was a monochromatic Al Kα (1486.6 eV) source operated at 100 W and 1 × 10^{-9} mbar. Fluorescence microscopy was realized using LD-A-Plan 10x/0.30 objective in Zeiss Axio Observer inverted microscope (ZEISS Fluorescence Microscopy, Carl Zeiss Canada Ltd, Canada). Filter set 38 (Excitation BP 470/40, Emission BP 525/50) was used for imaging of BODIPY-maleimide functionalized polymer brushes and filter set 43 (Excitation BP 545/25, Emission BP 605/70) for imaging of Qdot ® 605 immobilized polymers brushes. Obtained fluorescence images were processed by means of Zeiss AxioVision software. Elemental analysis data were obtained from Thermo Electron S.p.A. FlashEA® 1112 Elemental Analyzer (CHNS separation column, PTFE; 2 m; 6x5 mm).

Patterned polymer brushes were prepared by UV irradiation of ATRP initiator modified substrates using a transmission electron microscopy (TEM) grid as a photomask [48]. Silicon wafers were placed in a Novascan *PSD Series UV/Digital Ozone System* and irradiated for 30 min. Each initiated silicon surface was separated in two sections. One part of the surface was covered by another clean silicon wafer in order to protect it from UV irradiation. After surface-initiated ATRP, this section of the surface was utilized for XPS, water contact angle and ATR-FTIR analysis. On the other part of the substrate, a TEM grid was placed to form a micro-patterned area. In the regions which are located directly under UV light (with TEM grids) initiators were detached, whereas the areas covered with TEM grids stayed intact, enabling the polymerization. Polymerization occurred only in the regions protected by TEM grids. Patterned polymer brushes were prepared in this way.

4.3. Synthesis and Coating of the ATRP initiator

4.3.1. Synthesis of the ATRP initiator

The ATRP initiator was synthesized and coated on silicon surfaces according to previously reported literature procedure [46]. Briefly, 30 mL of CH_2Cl_2 was placed in a round bottom flask. Under N_2 atmosphere 5-hexen-1-ol (6.0 mL, 50 mmol) and subsequently triethylamine (7.0 mL) were added into the flask. The solution was stirred under N_2 and cooled with an ice bath. 2-bromo-2-methyl propionyl bromide (6.15 mL, 50 mmol) was added into the flask dropwise and slowly. The resulting mixture was stirred continuously and the reaction was let to continue under N_2 for 1 hour while ice bath was cooling (0 ºC). The reaction continued at room temperature for 3 hours, during which the solution turned milky white. The white coloured triethylammonium bromide precipitate, was filtered through 125 mm filter paper. The precipitate was washed several times with CH_2Cl_2 (150 mL). The filtrate was extracted with saturated NH_4Cl (3 × 15 mL). The yellowish organic phase was saturated with anhydrous Na_2SO_4 for drying and was filtered through a filter paper.

Then, the collected organic phase (100 mL) including the desired product was taken into a 250 mL RBF. The solvent CH_2Cl_2 was evaporated at 20 ºC. The yellowish product obtained was liquid. This crude product was then purified with column chromatography starting with 10:90 ethylacetate/hexane increasing to 25:75 ethylacetate/hexane. Thus, the pure 5-hexenyl-2-bromo-2-methyl propanoate product was obtained (m=13.28 g).

Furthermore, the obtained 5-hexenyl-2-bromo-2-methyl propanoate (3.0 mL,), was taken into a round bottom flask under N_2 atmosphere. Platinum on Carbon (Pt/C) was added as a catalyst (30 mg). Chlorodimethylsilane was added under N_2 (15.3 mL) and the solution was stirred for 24h at 50 ºC under reflux. Then, the mixture was filter off from celite Na_2SO_4 and washed with CH_2Cl_2 and the solvent was evaporate via rotary evaporator (3 mL was obtained). Distillation with a spider was realized to obtained the pure product at 270 ºC (1.33 g, yield: 27%).

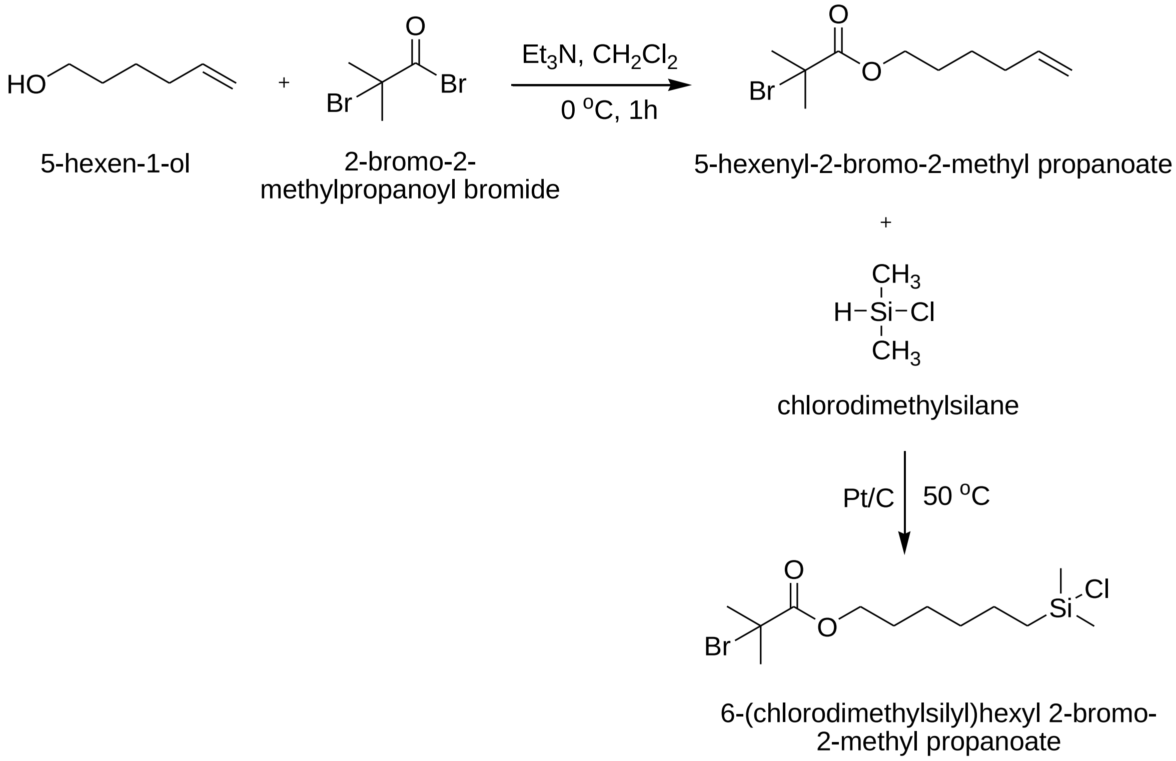


Figure 4.1. ATRP initiator synthesis.

4.3.2. Coating Si/SiO$_2$ Surfaces with ATRP Initiator

The Si/SiO$_2$ wafers were cleaned with H_2SO_4 and then with H_2O. Si/SiO$_2$ wafers were placed in 100 mL beaker and sonicated with acetone (20 mL) for acetone for 5 minutes. Acetone was poured and ethanol (20 mL) was added in the beaker and sonicated for another 5 minutes. Then, ethanol was poured and the Si/SiO$_2$ were washed with water (20 mL) for 5 minutes and finally rinsed with ethanol. The Si/SiO$_2$ wafers were placed into O_2 plasma for 10 minutes.

The Si/SiO$_2$ wafers were put in 50 mL round bottom flask which containing dry toluene (15 mL), N_2 baloon was placed at the top and sonicated for 5 minutes. The ATRP initiator was then added (30 μL) and the RBF was sonicated for 10 minutes. After waiting

24h, the Si/SiO_2 wafers were washed with CH_2Cl_2 (2 × 10 mL), sonicated with CH_2Cl_2 (1 × 10 mL) for 1 minute and washed with MeOH (2 × 10 mL). Then, they were dried via N_2 purge. Water contact angle of surface before coating was 30° and after coating with initiator was found to be 82°.

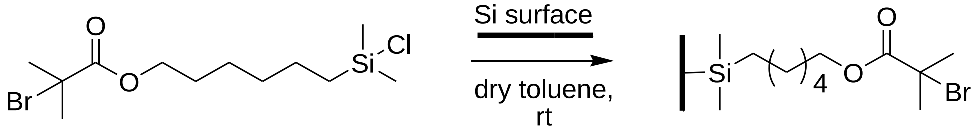


Figure 4.2. Modification of silicon surfaces with ATRP initiator.

4.4. Synthesis of Polymer Brushes via Surface Initiated – ATRP

Polymer brushes are synthesized via surface-initiated-ATRP. Three sets of brushes were synthesized containing different ratios of FuMA and PEGMEMA; 90:10 PEGMEMA/FuMA, 75:25 PEGMEMA/FuMA and 60:40 PEGMEMA/FuMA.

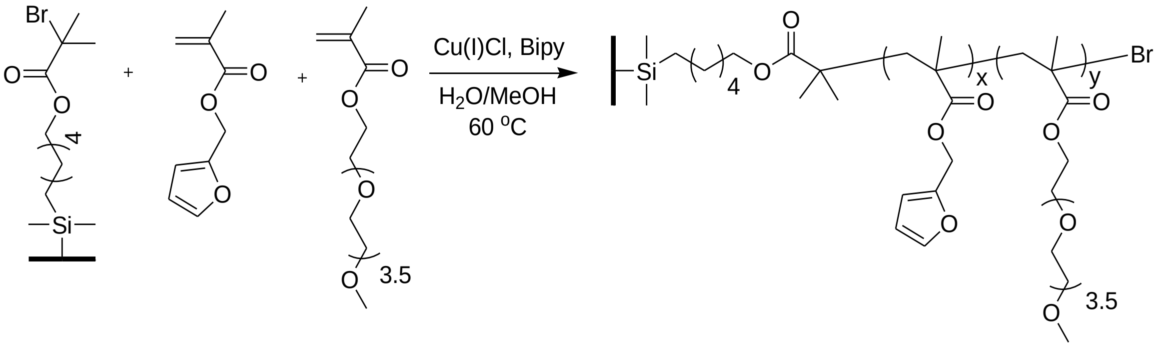


Figure 4.3. Polymer brushes synthesis via SI-ATRP.

4.4.1. Synthesis of Polymer Brushes with 90:10 PEGMEMA/FuMA

Cu(I)Cl (14.11 mg, 0.143 mmol), 2,2-bipyridine (62.91 mg, 0.403 mmol), were weighted into a round bottom flask and the flask was connected to a Schlenk line. Degassed FuMA (0.06 mL, 0.385 mmol), degassed PEGMEMA (0.99 mL, 3.465 mmol), degassed MeOH (0.36 mL), and degassed H_2O (0.4 mL) were added. This mixture was stirred for 15 minutes. The initiated Si/SiO_2 wafer was placed in a vial and purged under N_2. The mixture which containing the FuMA and PEGMEMA monomers was added on this silicon surface and the vial was placed in oil bath at 60 ºC. After polymerization for a specified time, the surface was washed with MeOH (3 × 2 mL) and H_2O (1 × 2 mL) and dried under a stream of a stream of N_2.

4.4.2. Synthesis of Polymer Brushes with 75:25 PEGMEMA/FuMA

Cu(I)Cl (14.11 mg, 0.143 mmol), 2,2-bipyridine (62.91 mg, 0.403 mmol), were weighted into a round bottom flask and the flask was connected to a Schlenk line. Degassed FuMA (0.15 mL, 0.96 mmol), degassed PEGMEMA (0.82 mL, 2.875 mmol), degassed MeOH (0.36 mL), and degassed H_2O (0.4 mL) were added. This mixture was stirred for 15 minutes. The initiated Si/SiO_2 wafer was placed in a vial and purged under N_2. The mixture which contained the FuMA and PEGMEMA monomers was added on this silicon surface and the vial was placed in oil bath at 60 ºC. After polymerization, the surface was washed with MeOH (3 × 2 mL) and H_2O (1 × 2 mL) and purged under N_2.

4.4.3. Synthesis of Polymer Brushes with 60:40 PEGMEMA/FuMA

Cu(I)Cl (14.11 mg, 0.143 mmol), 2,2-bipyridine (62.91 mg, 0.403 mmol), were weighted into a round bottom flask and the flask was connected to a Schlenk line. Degassed FuMA (0.24 mL, 1.535 mmol), degassed PEGMEMA (0.66 mL, 2.3 mmol), degassed MeOH (0.36 mL), and degassed H_2O (0.4 mL) were added. This mixture was stirred for 15 minutes. The initiator-coated Si/SiO_2 wafer was placed in a vial and purged under N_2. The mixture which containing FuMA and PEGMEMA monomers was added on this silicon surface and the vial was placed in oil bath at 60 ºC. After polymerization, the surface was washed with MeOH (3 × 2 mL) and H_2O (1 × 2 mL) and purged under N_2.

4.4.4. Characterization of Polymer Brushes

For all of the functionalizations, polymer brushes with 75:25 PEGMEMA/FuMA composition were used. Using techniques mentioned in section 4.2, all polymer brush coated surfaces were analyzed with AFM for thickness, goniometer for their water contact angle, and XPS for their chemical composition before and after functionalization.

4.5. Functionalization of Polymer Brushes

4.5.1. Functionalization of N-ethylmaleimide

5 mg/mL of N-ethylmaleimide in toluene was prepared as a stock solution. Polymer brushes containing furan groups in their structures were incubated in 1 mL of this stock solution for 16 hours. In order to realize the reverse reaction of Diels-Alder to obtain the polymer brushes at their original statues. N-ethylmaleimide functionalized polymer brush surfaces were dipped into 5 mL of toluene at 110 ºC for 16 hours.

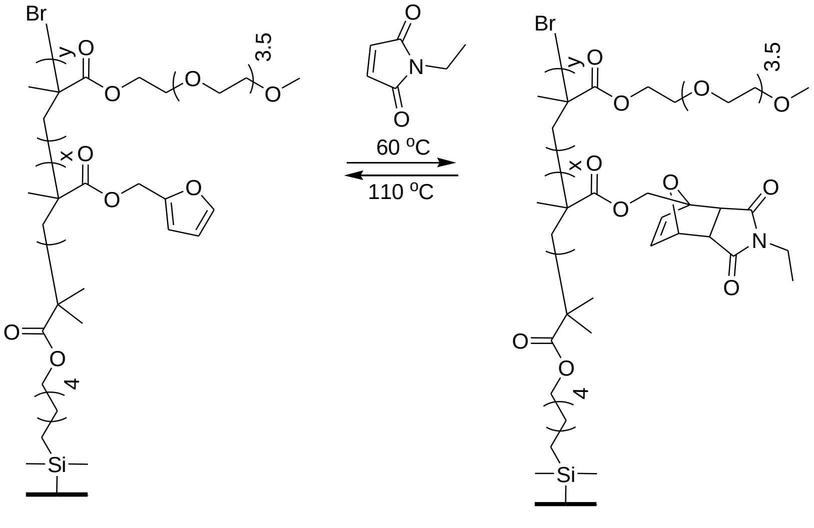


Figure 4.4. Attachment and detachment of N-ethylmaleimide on polymer brushes.

4.5.2. Synthesis of BODIPY-maleimide

4.5.2.1. Synthesis of the compound 2. In order to synthesize the compound 1, Diels-Alder reaction was realized between furan and maleic anhydride. Briefly, maleic anhydride (20 g, 203.96 mmol) was suspended in dry toluene (120 mL). The solution was heated at 80 °C under reflux. Then, furan (22.24 mL, 305.94 mmol) was added quickly to the solution under reflux and the mixture was stirred for 12 hours. After that, the reaction was led to cold and precipitated in cold toluene (40 mL). White crystals were formed. These crystals were filtered, washed with hexane (20 mL) and dried under vacuo to obtain the pure product (m=16.09 g, yield: 47%).

The compound 2 was synthesized according to the previously reported literature example [49]. Briefly, cyclo-adduct (2 g, 12 mmol) was added in 100 mL RBF containing 50 mL of CH_3OH. N_2 baloon was put at the top and the solution was stirred. N-propargylamine (1.07 g, 18 mmol) was added to another RBF containing CH_3OH (20 mL) and the solution was stirred under N_2 gas. The cyclo-adduct solution was cooled to 0 °C and the other solution, containing N-propargylamine, was added dropwise to this solution. The resulting mixture was stirred for 5 minutes at 0 °C and then for 30 minutes at room temperature. The mixture was finally placed in an oil bath at 65°C for 72 hours. After cooling the mixture to ambient temperature, the solvent was removed in rotary evaporator at 35°C. Then, CH_2Cl_2 (150 mL) was added to the residue was washed with aqueous solution (3 x 100 mL) and dried with Na_2SO_4. The residue was purified by column chromatography with 20:80 ethylacetate/hexane (33% yield).

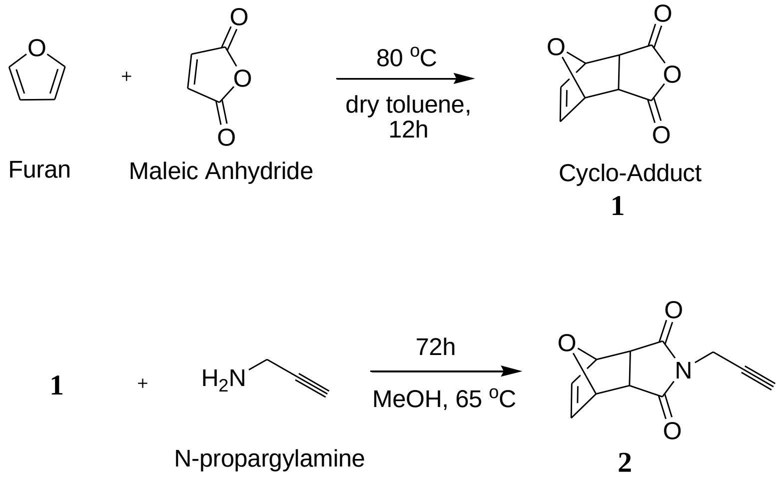


Figure 4.5. Synthesis of compound 2.

4.5.2.2. Synthesis of BODIPY-azide (3). BODIPY-azide was synthesized from the addition of sodium azide (NaN_3) on BODIPY-bromide [49]. BODIPY-bromide (354.7 mg, 0.763 mmol) was put in a RBF containing 9 mL of dry dimethylformamide (DMF). NaN_3 (496.03 mg, 7.63 mmol) was added in this RBF. This mixture was purged under N_2 and stirred for 16 hours at 60ºC. After wainting 16 hours, the solvent was evaporated via a rotary evaporator at 55ºC and then placed in high vacuum. CH_2Cl_2 (150 mL) was added to the remaining solid phase. The mixture was washed with H_2O (3 × 40 mL). Anhydrous Na_2SO_4 was added to the combined organic layers and concentrated after filtration and evaporation at 25ºC. The residue was purified by column chromatography with 5:95 ethylacetate/hexane to yield pure BODIPY-N_3 (0.147 g, yield: 44.7%).

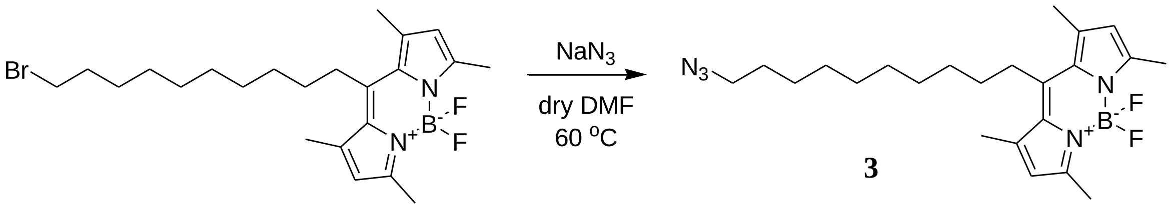


Figure 4.6. BODIPY-azide synthesis.

4.5.2.3. Synthesis of furan protected BODIPY-maleimide (4). Furan protected BODIPY-maleimide (4) was synthesized from "click" reaction between 2 and 3 according to the previously reported literature example [50]. In the first step, BODIPY-azide (130 mg, 0.302 mmol) and 2 (122.56 mg, 0.603 mmol) were dissolved in dry DMF (1.4 mL) and the mixture was stirred for 10 minutes under N_2 gas. Degassed Cu(I)Br (8.56 mg, 0.06 mmol) and degassed N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA) (12.5 μL, 0.06 mmol) were added to the mixture and stirred for 15 minutes. This mixture was immersed in an oil bath at 40ºC for 16 hours. Thereafter, the solvent was evaporated using a rotary evaporator. CH_2Cl_2 (150 mL) was added to the remaining solid phase. The mixture was washed with H_2O (3 × 40 mL) until blue color of aqueous phase was disappeared. Anhydrous Na_2SO_4 was added to the combined organic layers and concentrated after filtration and evaporation at 25ºC. The residue was purified by column chromatography with 50:50 ethylacetate/hexane to obtain the pure furan protected BODIPY-maleimide (m= 95.9 mg, yield: 50.3%) .

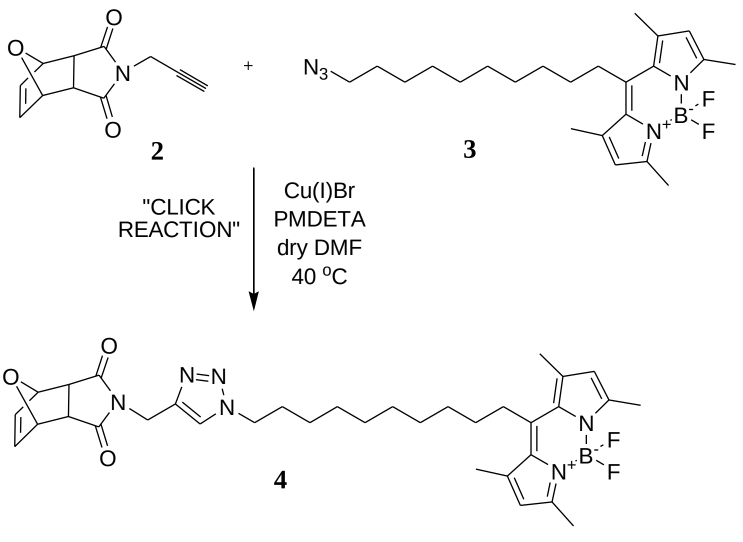


Figure 4.7. Furan protected BODIPY-maleimide synthesis.

4.5.2.4. Deprotection of furan protected BODIPY-maleimide (5). Furan protected BODIPY-maleimide was put in a RBF containing toluene (10 mL) and was refluxed at 110ºC. The solvent was evaporated and the residue was dissolved in ethylacetate (3 mL). The residue was purified by column chromatography with 30:70 ethylacetate/hexane. Thus, the pure BODIPY-maleimide product was obtained (m= 38.9 mg, yield: 87.2 %). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.5 (s, 1H), 6.6 (s, 2H), 6 (s, 2H), 4.75 (s, 2H), 4.25 (t, 2H), 2.9 (t, 2H), 2.5 (s, 6H), 2.39 (s, 6H), 1.85 (quintet J=7.8 Hz, 2H), 1.60 (m, 2H), 1.23-1.52 (broad, 12H).

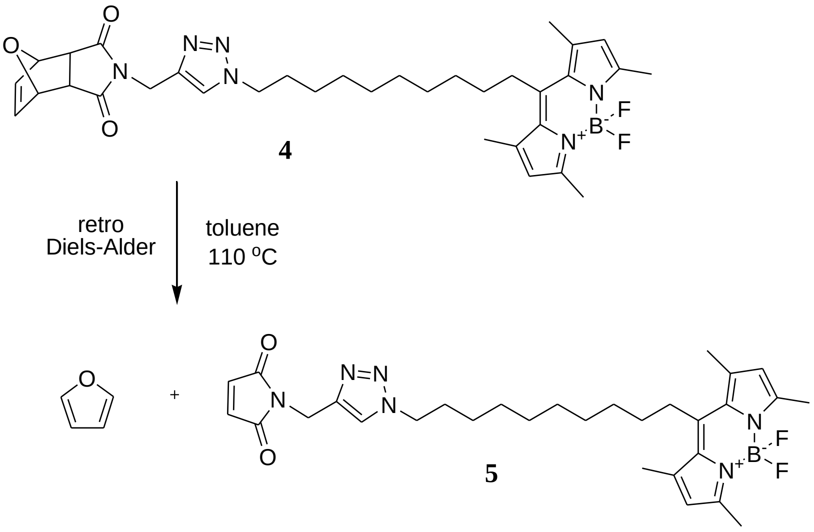


Figure 4.8. Deprotection of furan protected BODIPY-maleimide via retro-Diels-Alder.

4.5.3. Immobilization of BODIPY-maleimide

5 mg/mL of BODIPY-maleimide in toluene was prepared as a stock solution. Polymer brushes containing furan groups in their structures were incubated in 1 mL of this stock solution for 16 hours. In order to realize the reverse reaction of Diels-Alder to obtain the polymer brushes at their original statues. BODIPY-maleimide functionalized polymer brush surfaces were dipped into 5 mL of toluene at 110 ºC for 16 hours.

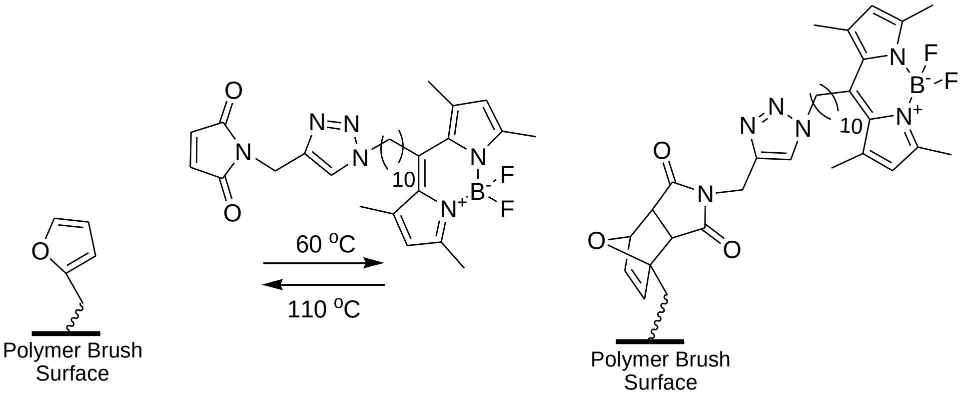


Figure 4.9. Attachment of BODIPY-maleimide.

4.5.4. Immobilization of Biomolecules

4.5.4.1. Functionalization with Biotin-maleimide. 2 mg/mL of biotin-maleimide in dimethylsulfoxide (DMSO) was prepared as a stock solution. Polymer brushes containing furan groups were incubated in 1 mL of this stock solution for 16 hours.

4.5.4.2. Immobilization of Streptavidin Conjugated Q-Dots to Biotinylated Brushes. Quantum dots (Q-Dots) streptavidin conjugate solution (5 µL) was diluted with distilled water (5 µL). This solution was dropped on patterned biotinylated polymer brush. After waiting 30 minutes, the surface was washed with a few pipettes of distilled water and purged under N_2 to dry.

5. CONCLUSION

In this study, novel reactive polymer brushes containing furan-based side chains were synthesized using surface-initiated ATRP. The pendant furan groups present in the polymer chains react with maleimide functionalized biomolecules via the Diels-Alder reaction. The specific characteristic of the biomolecule functionalization is allowed by the presence of the polyethylene glycol methyl ether methacrylate monomers in the polymer backbone. The “Diels-Alder” reaction is a convenient method because it is thermoreversible. In fact, in increased temperature environment the reverse reaction is done.

Several polymeric films, with different monomers ratios, are synthesized in order to study the reactivity of the furan groups. The efficient reactivity of furan containing brush surfaces are investigated with using different maleimide containing compounds, such as N-ethylmaleimide, BODIPY-maleimide fluorescent dye. N-ethylmaleimide attachment was analyzed by XPS and FT-IR. BODIPY-maleimide functionalization was observed with fluorescent microscopy. As a biomolecule model, biotin-maleimide was chosen to immobilize Quantum-Dots streptavidin conjugates.

APPENDIX A: SPECTROSCOPY DATA

^{1}H NMR, ATR-FTIR and XPS spectra of the synthesized compounds are included.

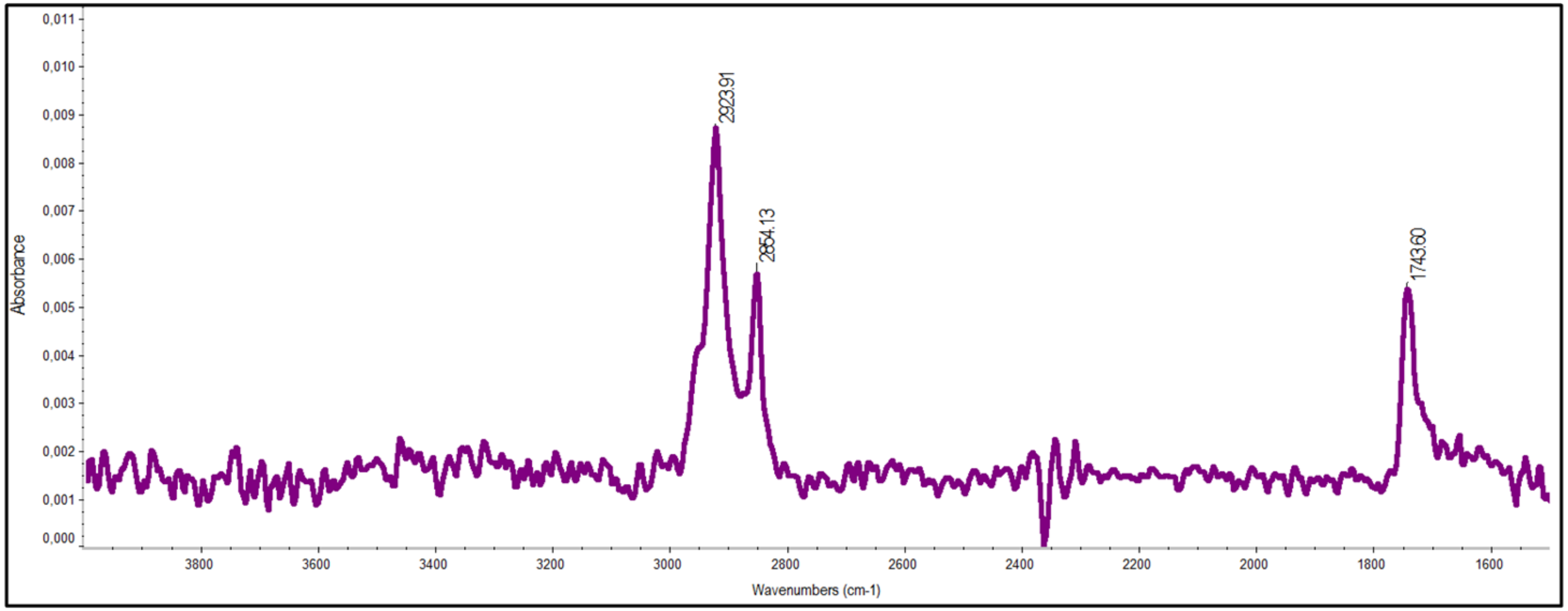


Figure A.1. ATR-FTIR spectrum of ATRP-initiator coated on silicon surface.

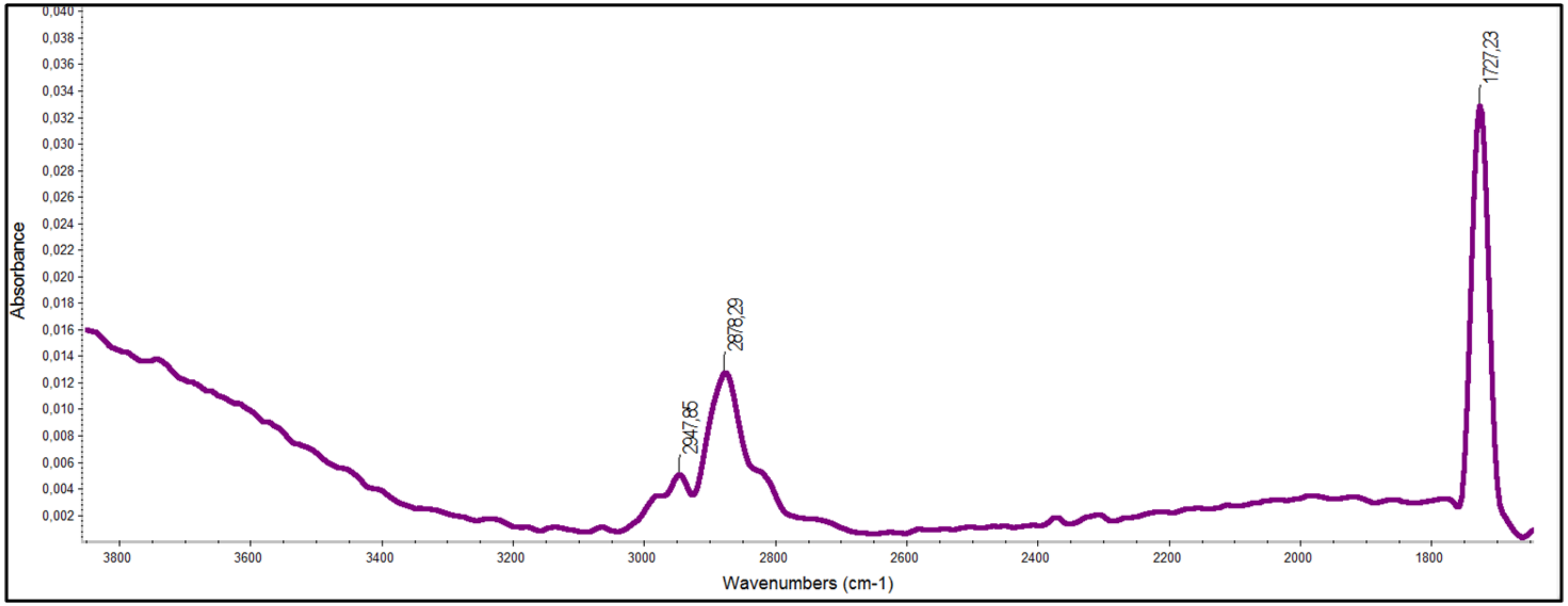


Figure A.2. ATR-FTIR spectrum of 100% PEGMEMA containing polymer brush surface.

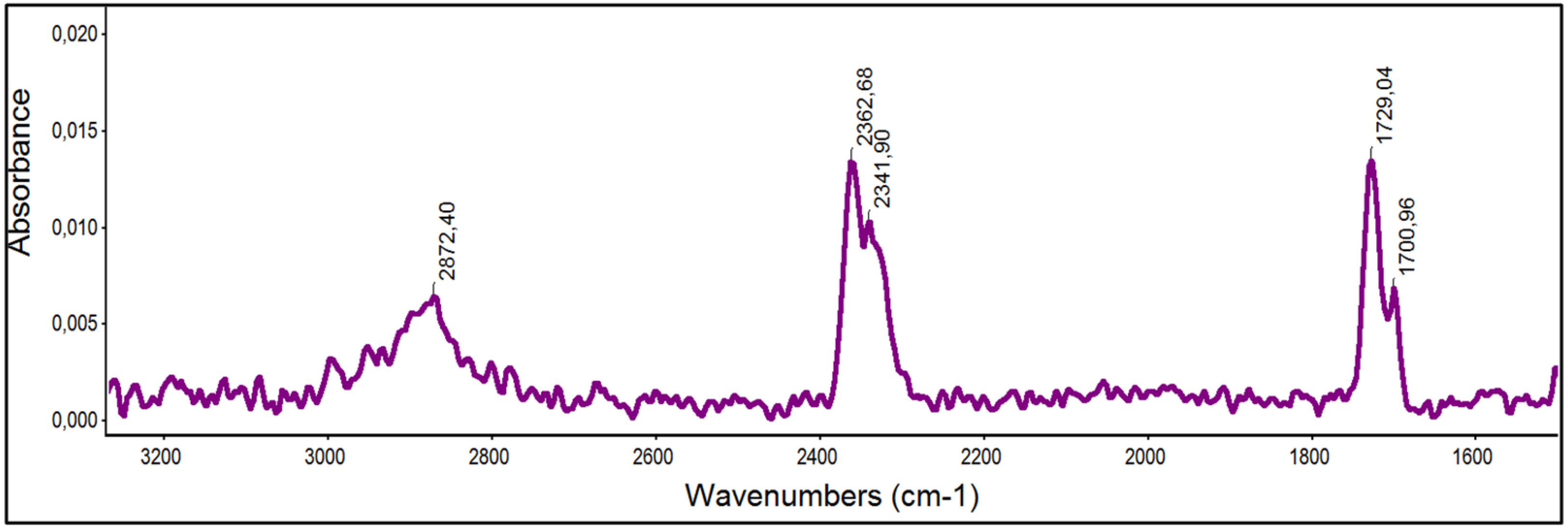


Figure A.3. ATR-FTIR spectrum of 10% FuMA containing polymer brush surface.

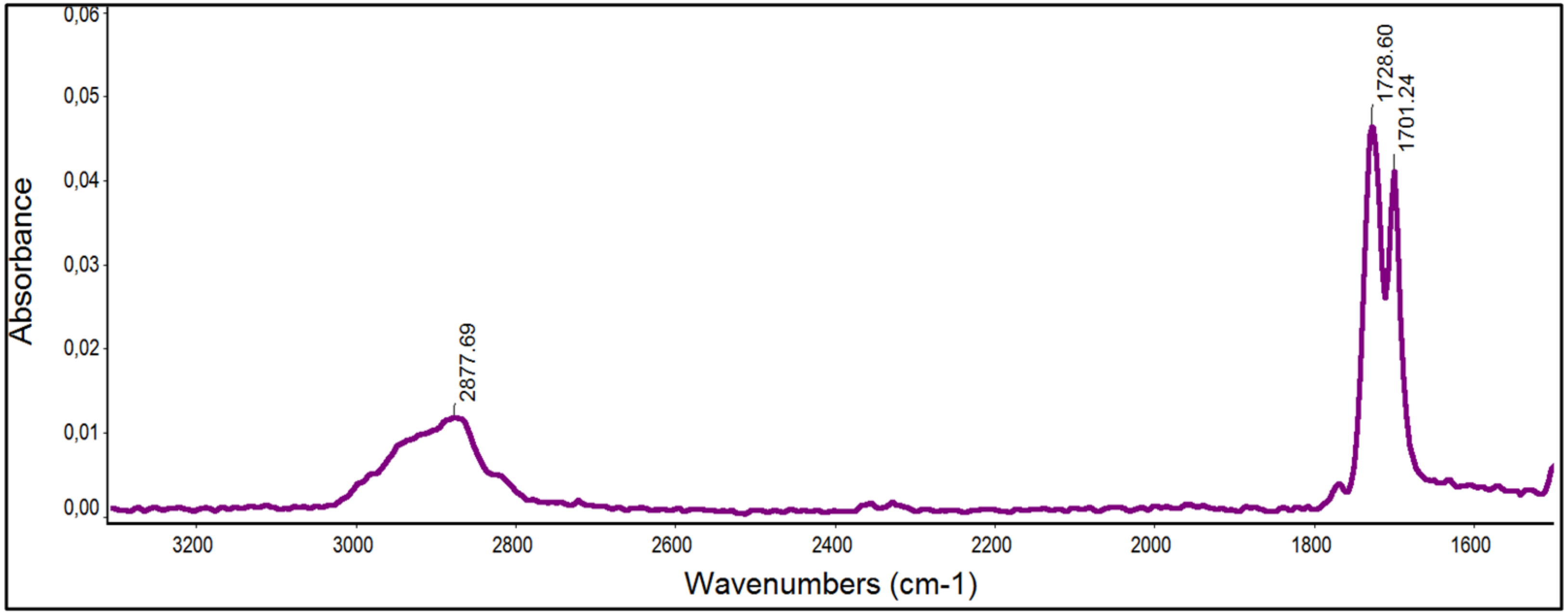


Figure A.4. ATR-FTIR spectrum of 25% FuMA containing polymer brush surface.

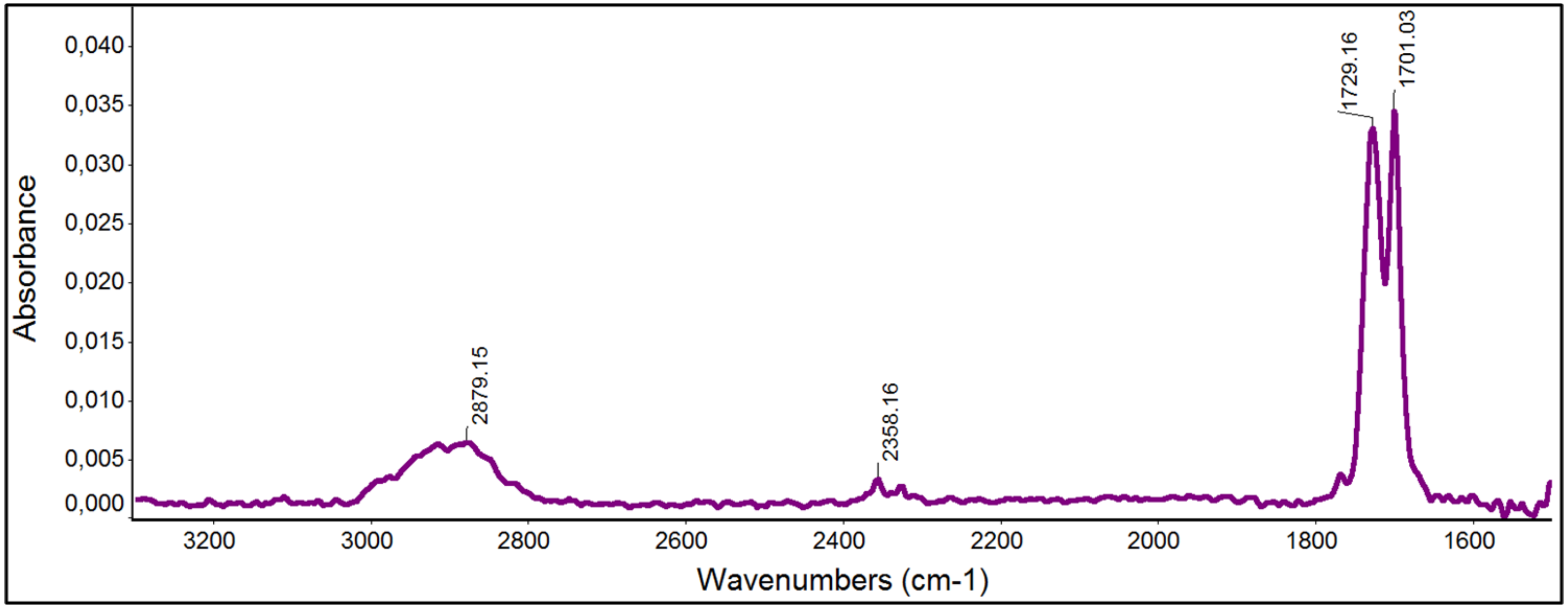
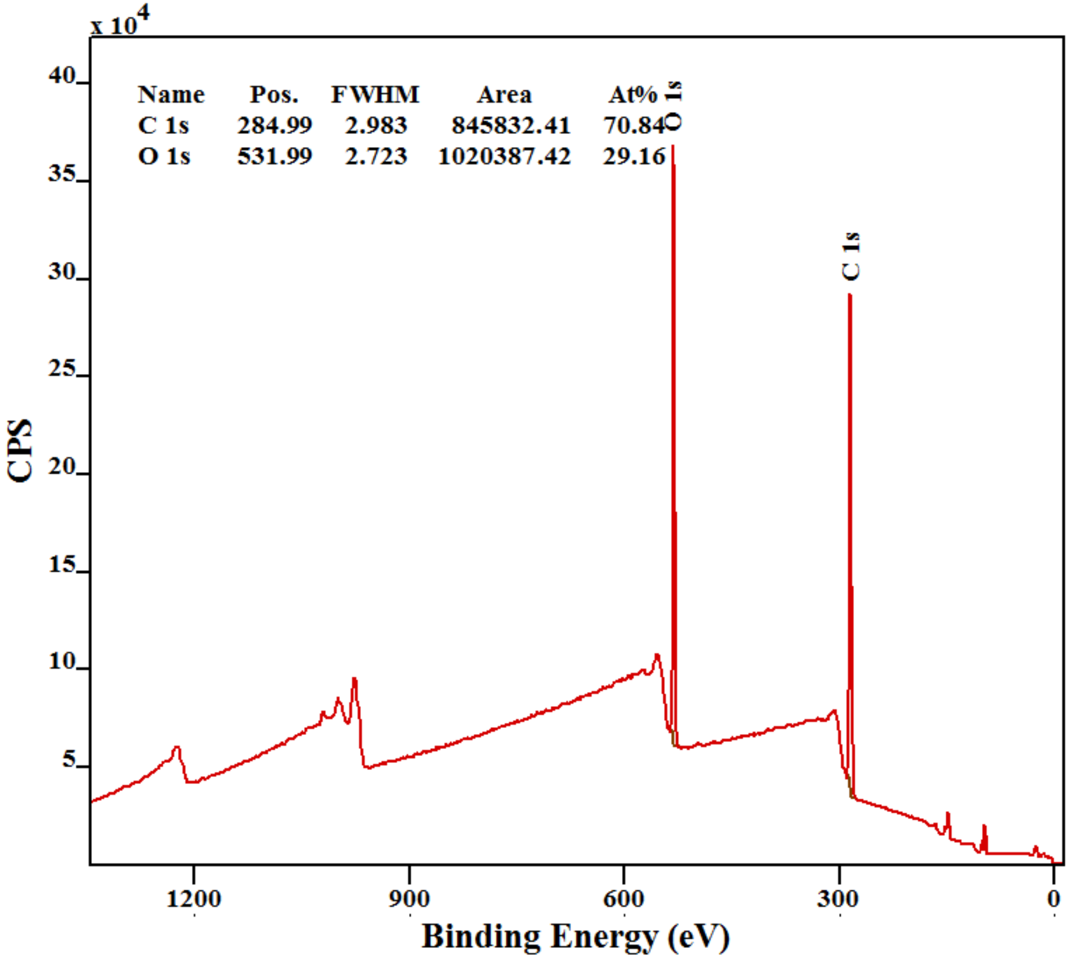
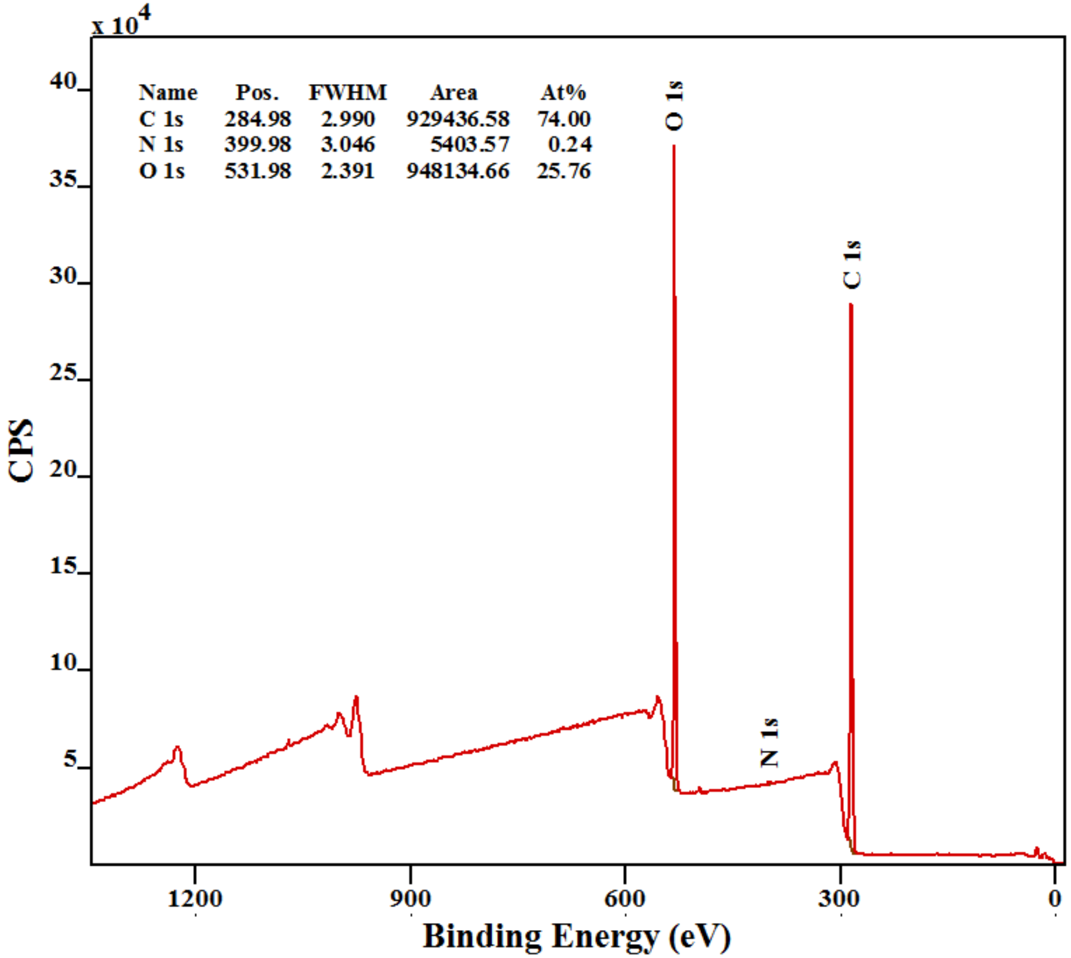


Figure A.5. ATR-FTIR spectrum of 40% FuMA containing polymer brush surface.



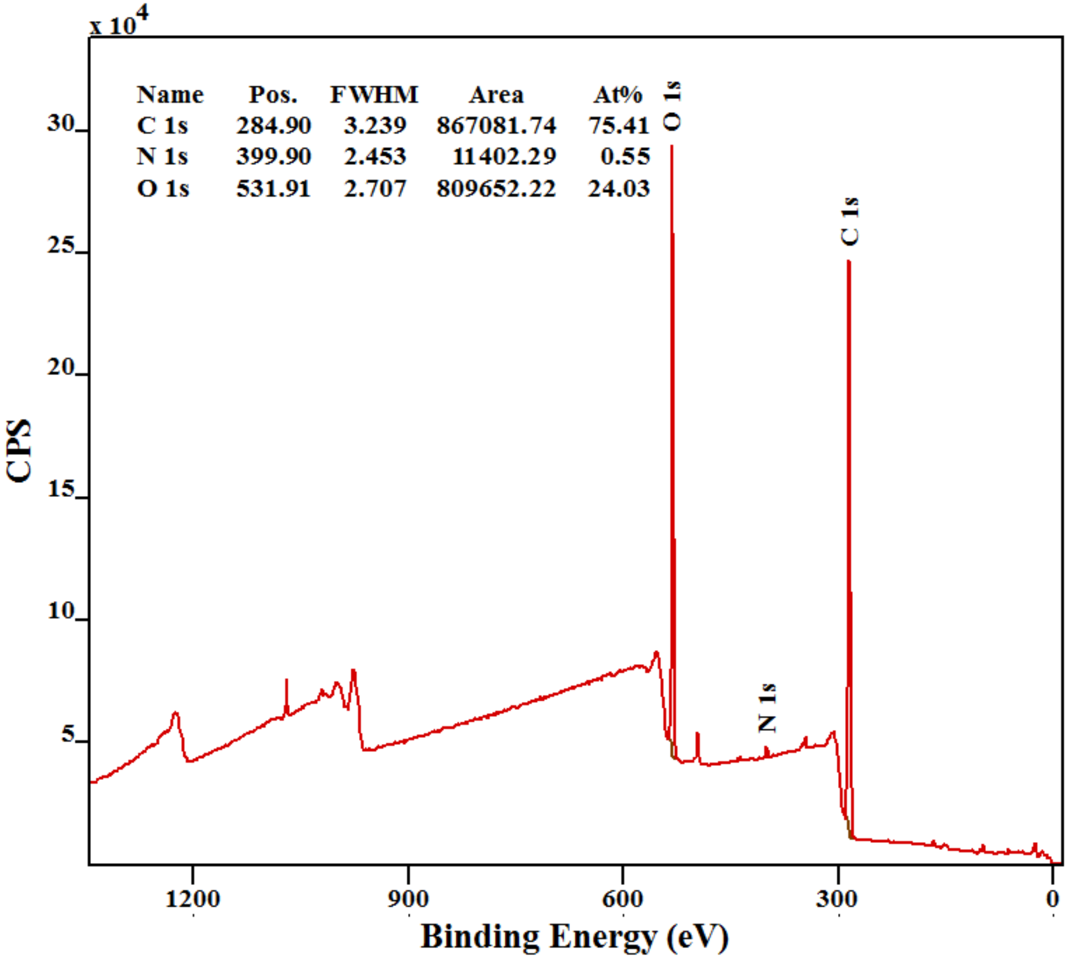
Name	Pos.	FWHM	Area	At%
C 1s	284.99	2.983	845832.41	70.84
O 1s	531.99	2.723	1020387.42	29.16

Figure A.6. XPS survey of 100% PEGMEMA containing polymer brush surface.



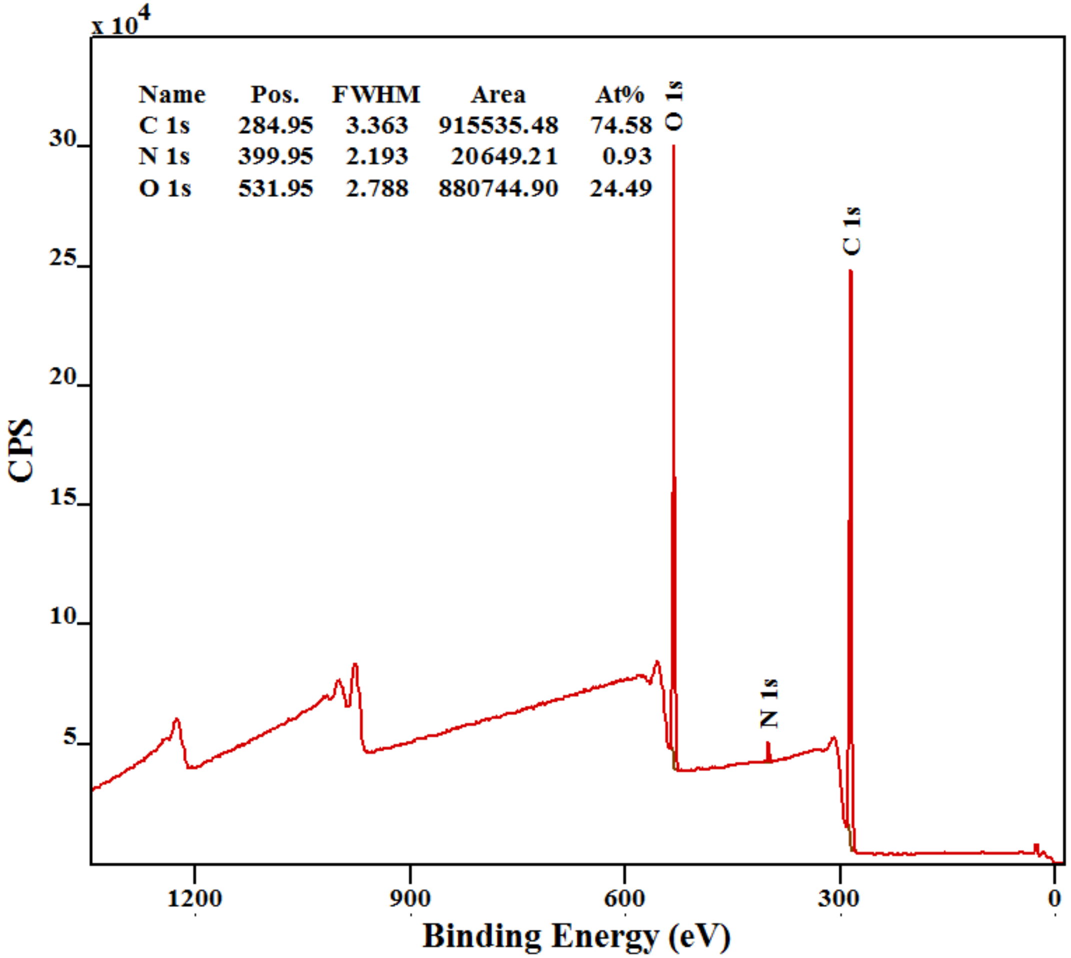
Name	Pos.	FWHM	Area	At%
C 1s	284.98	2.990	929436.58	74.00
N 1s	399.98	3.046	5403.57	0.24
O 1s	531.98	2.391	948134.66	25.76

Figure A.7. XPS survey of 10% FuMA containing polymer brush surface.



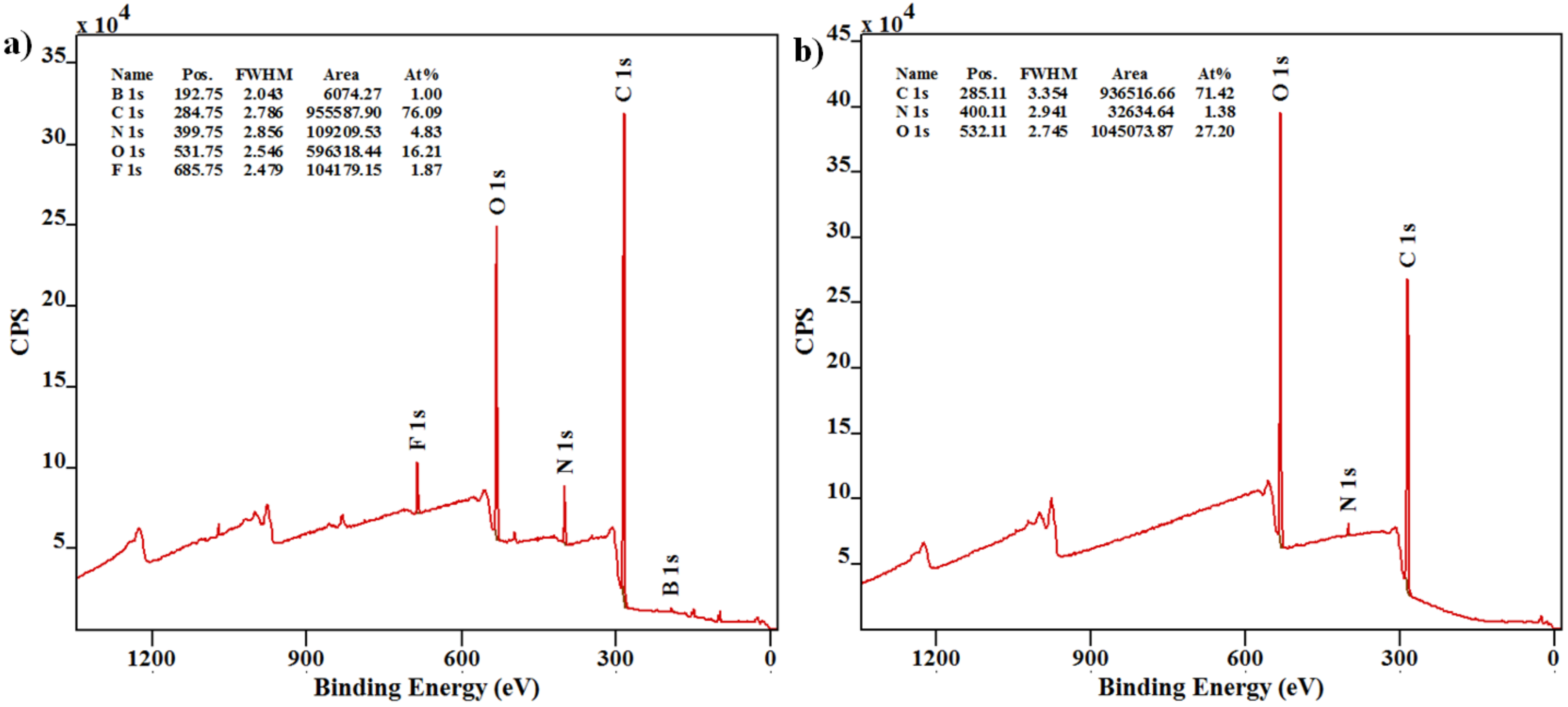
Name	Pos.	FWHM	Area	At%
C 1s	284.90	3.239	867081.74	75.41
N 1s	399.90	2.453	11402.29	0.55
O 1s	531.91	2.707	809652.22	24.03

Figure A.8. XPS survey of 25% FuMA containing polymer brush surface.



Name	Pos.	FWHM	Area	At%
C 1s	284.95	3.363	915535.48	74.58
N 1s	399.95	2.193	20649.21	0.93
O 1s	531.95	2.788	880744.90	24.49

Figure A.9. XPS survey of 40% FuMA containing polymer brush surface.



a)

Name	Pos.	FWHM	Area	At%
B 1s	192.75	2.043	6074.27	1.00
C 1s	284.75	2.786	955587.90	76.09
N 1s	399.75	2.856	109209.53	4.83
O 1s	531.75	2.546	596318.44	16.21
F 1s	685.75	2.479	104179.15	1.87

b)

Name	Pos.	FWHM	Area	At%
C 1s	285.11	3.354	936516.66	71.42
N 1s	400.11	2.941	32634.64	1.38
O 1s	532.11	2.745	1045073.87	27.20

Figure A.10. XPS survey of BODIPY-maleimide functionalized poly(PEGMEMA-*ran*-FuMA brush, a) before retro-Diels-Alder and b) after retro-Diels-Alder.

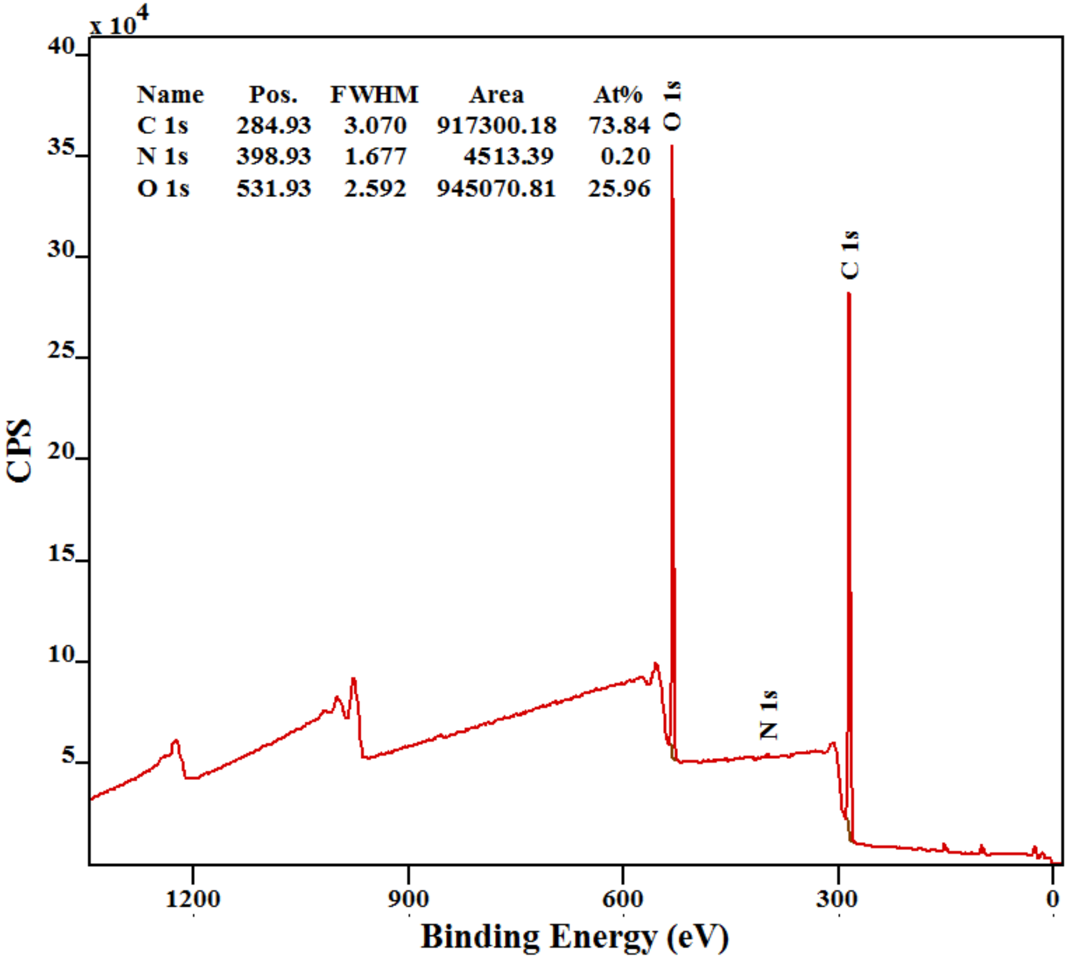


Figure A.11. XPS survey of biotin-maleimide functionalized poly(PEGMEMA-*ran*-FuMA brush.

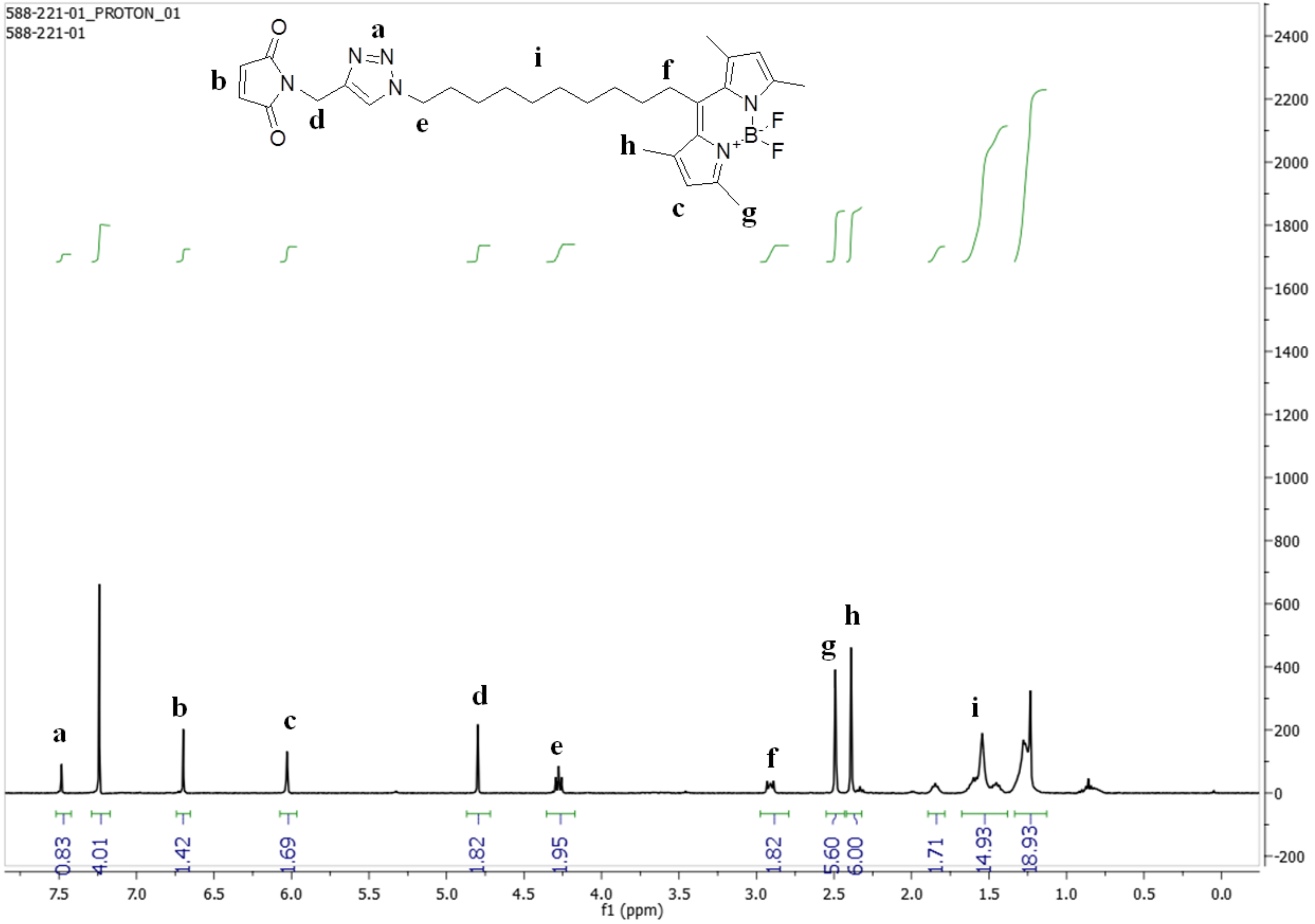


Figure A.12. ^{1}H NMR spectrum of BODIPY-maleimide.

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