

# Polyelectrolyte Multilayers with Tunable Properties - Synthesis, Layer Assembly, and Post-modification

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Publication date: 2020

Document Version Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

Jiang, T. (2020). Polyelectrolyte Multilayers with Tunable Properties - Synthesis, Layer Assembly, and Postmodification. Technical University of Denmark.

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Polyelectrolyte Multilayers with Tunable Properties - Synthesis, Layer Assembly, and Post-modification





# Polyelectrolyte Multilayers with Tunable Properties - Synthesis, Layer Assembly, and Post-modification

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Ph.D. Thesis

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# Abstract

Layer-by-layer (LbL) assembly is a highly versatile approach to the fabrication of thin films for surface modification. The polyelectrolyte multilayers (PEMs) prepared accordingly has been extensively studied, demonstrating tremendous potential in a wide range of applications. The functionality of a PEM film is dependent on the physicochemical properties of the film, which are determined by its chemical composition and the internal structure. This PhD thesis aims to raise strategic approaches to systematically tune the physicochemical properties of a PEM film by utilization of tailor-designed synthetic polyelectrolytes and post-assembly chemical modification. This PhD work in total involves four studies, two published journal articles, one manuscript, and one unpublished study.

In the first study (Paper 1), I proposed a novel approach to systematically tune the growth mechanism and water content of a PEM film by incorporation of PEGMEMA-based copolymers with variable PEG side chain lengths. To do so, I copolymerized poly (ethylene glycol) methyl ether methacrylate (PEGMEMA) with a charge-bearing co-monomer, 2-aminoethyl methacrylate (AMA). PEGMEMA-based polycations were obtained and successfully co-deposited with alginate by LbL assembly. Upon increasing the PEG side chain length, the PEM film demonstrated a shift in growth mechanism, as well as an increased water content. In addition, the film was stabilized against disintegration upon pH variation by partial cross-linking of the amino groups with the treatment of glutaraldehyde. Finally, the cross-linked PEM film was found to show a pH-responsive behavior, exhibiting cationic, zwitterionic, and anionic charging states as a function of the pH value, associated with a film contraction and swelling behavior, as well as water content changes.

In the second study (Paper 2), I raised an approach of fabricating a single-component, cross-linked, and surface-grafted polyelectrolyte thin film with tunable layer thickness. In this regard, I expanded the concept of copolymerization in Paper 1 and prepared a PEGMEMA-based polyanion, which was co-deposited with the PEGMEMA-based polycation in an LbL assembly process. After cross-linking with EDC/NHS, the excess amino groups in the film were quenched to provide a

single-component anionic PEGMEMA-based polyelectrolyte layer. The obtained polyelectrolyte layer showed an enhanced BSA repellence compared to the bare substrate and the zwitterionic film before amine quenching.

While the first two studies emphasized the bulk properties and the internal, the third study (Paper 3) focused on tuning the interfacial property of the PEM film. I successfully prepared a PEM film comprising PAMA and polymethacrylic acid (PMAA) with reactive amino groups situated selectively in the outer layer. Therefore, a selective tuning of the chemical composition of the outer layer is achieved by a conjugation reaction with functionalized carboxylic acids catalyzed by EDC/NHS. As an illustrative example, I successfully grafted undecanoic acid and m-PEG3-COOH, onto the outer layer of the film, obtaining two polyelectrolyte films showing different surface hydrophilicity but a similar pH-responsiveness.

Finally, the fourth study was to further investigate the surface interaction of the two PEM films prepared by colloidal probe atomic force microscopy (CP-AFM) using a hydrophobic colloidal probe. The adhesion force and energy were examined with three varying parameters, namely the surface hydrophilicity, pH value, and the effect of a multivalent cation ( $Ca^{2+}$ ). The pH-responsive behavior of the films was confirmed in the force measurement, following the same trend as observed in paper 3 with QCM-D. The film modified with undecanoic acid showed a higher adhesion force, which follows our hypothesis that the strong hydrophobic-hydrophobic interaction facilitates the adhesion. However, it was expected that the film modified with undecanoic acid show high adhesion energy at pH 9 in the presence of Ca2+. This trend was not observed, which was attributed to the relatively low grafting density of the hydrophobic alkyl chains.

# Danske Resumé

Lag-for-lag (LbL) samling er en meget alsidig tilgang til fremstilling af tynde film til overflademodificering. Polyelektrolyt-multilagene (PEM'er), der er fremstillet i overensstemmelse hermed, er blevet undersøgt omfattende, hvilket demonstrerer et enormt potentiale i en lang række anvendelser. Funktionen af en PEM-film er afhængig af filmens fysisk-kemiske egenskaber, der bestemmes af dens kemiske sammensætning og dens interne struktur. Denne ph.d.-afhandling har til formål at skabe strategiske tilgange til systematisk at justere de fysisk-kemiske egenskaber ved en PEM-film ved hjælp af skræddersyede syntetiske polyelektrolytter og kemisk modifikation efter samlingen. Dette ph.d.-arbejde involverer i alt fire studier, to artikler, et manuskript, og en upubliceret undersøgelse.

I den første undersøgelse (Paper 1) foreslog jeg en ny tilgang til systematisk at justere vækstmekanismen og vandindholdet i en PEM-film ved inkorporering af PEGMEMA-baserede copolymerer med variabel PEG-sidekædelængde. For at gøre dette copolymeriserede jeg poly (ethylene glycol) methyl ether methacrylate (PEGMEMA) med en ladningsbærende co-monomer, 2-aminoethyl methacrylate (AMA). PEGMEMA-baserede polykationer blev opnået og co-deponeret succesfuld med alginat ved LbL-samling. Efter øget PEG-sidekædelængde demonstrerede PEM-filmen en forskydning i vækstmekanismen såvel som et forøget vandindhold. Derudover blev filmen stabiliseret mod desintegration efter pH-variation ved partiel tværbinding af aminogrupperne med behandlingen af glutaraldehyd. Endelig blev det fundet, at den tværbundne PEM-film udviste en pH-responsiv adfærd, udviste kationiske, zwitterioniske og anioniske ladningstilstande som en funktion af pH-værdien, der er forbundet med en filmkontraktion og hævelse, samt ændringer i vandindhold.

I den anden undersøgelse (Paper 2) brugte jeg en fremgangsmåde til fremstilling af en enkeltkomponent, tværbundet og overfladetransporteret tynd film med elektrolektrolyt med justerbar lagtykkelse. I denne henseende udvidede jeg begrebet copolymerisation i Paper 1 og forberedte en PEGMEMA-baseret polyanion, som blev co-deponeret med den PEGMEMA-baseret polyanion, i en LbL-samlingsproces. Efter tværbinding med EDC/NHS blev

overskydende aminogrupper i filmen standset for at tilvejebringe et enkeltkomponent anionisk PEGMEMA-baseret polyelektrolytlag. Det opnåede polyelektrolytlag udviste en forbedret BSAekspertise sammenlignet med det blotte underlag og den zwitterioniske film før amin-slukning.

Mens de to første undersøgelser lægger vægt på bulkegenskaberne og de interne egenskaber, fokuserede den tredje undersøgelse (Paper 3) på at indstille PEM-filmens grænsefladeegenskaber. Jeg fremstillede med succes en flerlagsfilm omfattende PAMA og poly(methacrylic acid) (PMAA) med reaktive aminogrupper, der var selektivt placeret i det ydre lag. Derfor opnås en selektiv indstilling af den kemiske sammensætning af det ydre lag ved en konjugeringsreaktion med funktionaliserede carboxylsyrer katalyseret af EDC / NHS. Som et illustrerende eksempel podede jeg med succes undekanoinsyre og m-PEG3-COOH på det ydre lag af filmen, hvilket opnåede to polyelektrolytfilm, der viser forskellig overfladehydrofilicitet, men en lignende pH-reaktionsevne.

Endelig var den fjerde undersøgelse yderligere at undersøge overfladeinteraktionen af de to PEMfilm, der blev fremstillet ved kolloidal probe-atomkraftmikroskopi (CP-AFM) under anvendelse af en hydrofob kolloid probe. Adhæsionsstyrken og energien blev undersøgt med tre forskellige parametre, nemlig overfladens hydrofilicitet, pH-værdi og virkningen af en multivalent kation (Ca<sup>2+</sup>). Den pH-responsive opførsel af filmene blev bekræftet ved kraftmåling efter den samme tendens som observeret i Paper 3 med QCM-D. Filmen modificeret med undekansyre viste en højere vedhæftningskraft, som følger vores hypotese om, at den stærke hydrofobe-hydrofobe interaktion mindsker adhæsionen. Det blev imidlertid forventet, at filmen, der var modificeret med undecanoinsyre, udviser høj vedhæftningsenergi ved pH 9 i nærværelse af Ca<sup>2+</sup>. Denne tendens blev ikke observeret, hvilket blev tilskrevet den relativt lave podningstæthed af de hydrofobe alkylkæder.

# Acknowledgments

First of all, I would like to express my sincere gratitude to my principal supervisor, Professor Esben Thormann, without whose tremendous support this work could have never become a reality. Esben advised me when I lacked academic inspiration, encouraged me when I suffered setbacks in research, and pushed me when I lacked motivation. Moreover, his positivity and optimism has not only encouraged me throughout the past three years but will continue to inspire me in tackling future challenges in my life.

I would also like to thank my co-supervisor, Dr. Saeed Zajforoushan Moghaddam, for his insightful discussions on my project, his patient instructions concerning instrumentations, as well as his constructive comments regarding academic writing. Saeed is nice, talented, and professional, and is always a role model.

I would like to express my appreciation to all the colleagues, both former and current, in the GPFI group (Xiaoyan Liu, Roberto Ortiz, Junhao Huang, Daniel Hansen, Fatemeh Keshavarzi, Johannes Eiler, Frederik Hegaard, Robert Biro, Junjie Kang, Runtian Qie, Koosha Ehtiati, Yun Bai, Gokce Engudar, Wenbo Wang, Niloufar Komjani, and Khorshid Kamguyan). Thanks for making GPFI a warm family, where I always feel like home. The moment with all of you has been fun and inspiring, which is worth a lifetime memory.

I gratefully acknowledge the financial support from the Independent Research Fund Denmark, as well as all the technical and administrative support from DTU chemistry. It has been an honor and a pleasure to work in such a sweet and supportive department. Special thanks to the coffee machine in the ground-floor canteen of Building 206, which has greatly boosted my academic productivity.

Finally, I owe thanks to my family, for always standing by my side throughout the toughest times. I owe thanks to my "kæreste" Huaiqing, for being the sunshine in my life throughout the journey.

# List of Abbreviations

AF4	Asymmetric flow field flow fractionation
APTES	(3-Aminopropyl)triethoxysilane
ATRP	Atom transfer radical polymerization
BEMA	Bruggeman effective medium approximation
Boc	tert-Butyloxycarbonyl
bPEI	Branched polyethylenimine
BSA	Bovine serum albumin
CP-AFM	Colloidal probe atomic force microscopy
DCM	Dichloromethane
DMF	Dimethylformamide
dRI	Differential refractive index
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide)
НА	Hyaluronic acid
LbL	Layer-by-layer
MALS	Multiangle light scattering
Me6TREN	Tris[2-(dimethylamino)ethyl]amine
MWCO	Molecular weight cut off
NHS	N-Hydroxysuccinimide
NMR	Nuclear magnetic resonance
PAA	Poly(acrylic acid)
РАН	Poly(allylamine hydrochloride)
PAMA	Poly(2-aminoethyl methacrylate)
PDI	Polydispersity index
PEG	Polyethylene glycol
PEGMEMA	Poly(ethylene glycol) methyl ether methacrylate

PEM	Polyelectrolyte multilayer
PGA	Polyglutamic acid
PHEMA	Polyhydroxyethylmethacrylate
PLL	Polylysine
РМАА	Poly(methacrylic acid)
PMDETA	(N,N,N',N'',N'' -pentamethyldiethylenetriamine)
PMeDMA	Poly([2-(methacryloyloxy)ethyl]trimethylammonium chloride)
PolyDADMAC	Poly(diallyldimethylammonium chloride)
PSS	Poly(styrene sulfonate)
PVS	Poly(vinyl sulfonate)
QCM-D	Quartz crystal microbalance with dissipation monitoring
SEC	Size exclusion chromatography
TFA	Triflouroacetic acid

# List of Publications

My PhD work is based on the following publications.

- PPEGMEMA-Based Cationic Copolymers Designed for Layer-by-Layer Assembly. Tao Jiang, Saeed Zajforoushan Moghaddam, Esben Thormann. *RSC Adv.* 2019, 9, 26915–26926.
- A Single-component Cross-linked and Surface Grafted Polyelectrolyte Film Fabricated by a Layer-by-layer Assembly Process.
  Tao Jiang, Saeed Zajforoushan Moghaddam, Esben Thormann.
  Polymer. 2020, 200, 122524
- 3. A cross-linked and pH-responsive Polyelectrolyte Multilayer Film with Tunable Interfacial Properties

Tao Jiang, Saeed Zajforoushan Moghaddam, Esben Thormann. To be submitted

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# 1 Background

## 1.1 Polyelectrolytes

A polyelectrolyte is defined as a polymer possessing ionizable repeating units. Due to its highly charged nature, most polyelectrolytes exhibit high hydrophilicity and good solubility in aqueous solutions. Depending on whether the repeating units carry positive, negative, or simultaneously both types of charges, a polyelectrolyte can be categorized as either a polycation, a polyanion, or a polyampholyte, respectively.[1] Based on the nature of the ionizable groups in aqueous solution, polyelectrolytes can be classified as strong polyelectrolytes and weak polyelectrolytes.[1] A strong polyelectrolyte comprises strong electrolyte repeating units that are ionized completely in solution in a wide range of pH. For weak polyelectrolytes, the major charging species in solution is a weak electrolyte, and the degree of ionization and hence the charge density depends significantly on the pH value of the solution.



Figure 1.1 The structures of some commonly used polyelectrolytes

Unlike non-charged polymers, polyelectrolytes exhibit a conformation as a function of the ionic strength in aqueous solutions.[2] In solutions with low salt concentration, linear polyelectrolytes tend to adopt a stretched and "rod-like" conformation, due to the intramolecular electrostatic repulsion between the charged repeating units. However, in solutions with a high salt concentration, the polyelectrolyte chains adopt a more shrunk and coiled conformation due to the screening effect of the salt ions. In addition, a polyelectrolyte chain is coupled with free ions of the opposite charge, or the so-called "counterions", in an aqueous solution. While some of the counterions can freely detach from the polyion, a significant proportion of the counterions are bounded to the PE chain to reduce the charge density of the chain below a critical value. This ion-binding effect, or the counterion condensation phenomenon, was described by the Oosawa-Manning theory[3–6] and the Poisson-Boltzmann theory.[7,8]

Based on the origin, a polyelectrolyte can be classified as a natural or semi-natural polyelectrolyte or a synthetic polyelectrolyte.[9] The advantages of natural and semi-natural polyelectrolytes include natural abundance, good biocompatibility, and sustainability. However, compared to synthetic polyelectrolytes, there lacks a precise control and regulation regarding chemical composition and architecture. This work places a major emphasis on synthetic polyelectrolytes. In particular, a series of copolymer-type polyelectrolytes were designed and synthesized as components for PEM film fabrication.

## 1.2 Polyelectrolyte Multilayers

### 1.2.1 Layer-by-layer (LbL) Assembly & PEM Films

Layer-by-layer (LbL) assembly has been widely regarded as an easy, flexible, and versatile method for thin-film fabrication. The concept of sequential adsorption of two species of materials carrying opposite charges onto a substrate dates back to 1966 when Iler et al. prepared a multilayered film comprising particles with opposite charges.[10] It was not until when Decher published his pioneering works in the 1990s,[11,12] that the significance of LbL assembly was valued. Since then LbL assembly has been widely explored due to its high versatility compared to other surface modification techniques.[13–16] A wide range of layer materials has been reported in LbL assembly, including polymers, nanoparticles,[17] peptides,[18] nucleic acids,[19] and so on. In addition, LbL can be performed on a variety of different substrates (planar/curved surfaces, nanoparticles,[20] fibers,[21] membranes[22]) with various coating techniques (dip-coating, spray

coating, spin coating[23,24]). Meanwhile, the physicochemical properties of the fabricated film (film thickness, net charge, film viscoelasticity, and so on) is easily tunable by controlling the polyelectrolyte chemical composition, deposition conditions, and the coating techniques. For this reason, the LbL assembly technique is considered promising in applications including biosensing, drug delivery, cell growth/differentiation, catalysis, energy storage and conversion, etc. [15,16]



*Figure 1.2* Schematic illustration of PEM film fabrication by LbL assembly

A polyelectrolyte multilayer (PEM) film is fabricated by LbL assembly of a polycation and a polyanion onto a charged substrate driven by the intermolecular electrostatic interaction. (Figure 1.2) In-situ polyelectrolyte complexation occurs as one polyelectrolyte species deposits on top of the other bearing the opposite charge. Through the polyelectrolyte complexation process, the paring of opposite charges in between polyelectrolytes drives the release of the bounded counterions and affiliated water molecules. A significant entropy gain is resulted accordingly, serving as the major driving force for the complexation and LbL assembly.[25,26] Accordingly, ionic cross-linking, or "intrinsic" compensation, forms inside the PEM film, preventing the whole layer from disintegration. Meanwhile, the charges in the PEM film remain also partially

compensated by counterions in the solution, forming the so-called "extrinsic" compensation.[27,28]

The ionic cross-linking strength and density are crucial to the film's physicochemical properties.[29,30] Generally, a higher ionic cross-linking density, or higher ratio of intrinsic compensation, leads to thinner, smoother, and more rigid film. In addition, the film normally exhibits weak swelling behavior in aqueous conditions and has low water content. On the other hand, a lower ionic cross-linking density, or higher ratio of extrinsic compensation, results in a thicker, rougher, and softer film. The film assembled accordingly demonstrates relatively high swelling and hydration levels. The ionic cross-linking in the film is influenced by a range of parameters including the charge density of the polyelectrolyte, the chemical composition, and the deposition condition of the LbL assembly.[13,30–32] This will be elaborated in later sections.

The LbL assembly can be monitored with a range of surface characterization techniques, the most commonly reported ones including quartz crystal microbalance (QCM),[33–35] spectroscopic ellipsometry,[36,37] neutron and X-ray reflectivity,[38–41] UV-vis,[42,43] and surface plasmon resonance.[44,45] In this work, I utilized a combined QCM-D and ellipsometry measurement for characterizing the layer growth of the PEM film. This combined technique allows for simultaneous in-situ monitoring of deposited mass, film thickness, film optical constants, and hydration level.

#### 1.2.2 LbL Growth Mechanisms

There are two types of film growth mechanisms, namely the linear growth and exponential growth. In the linear growth regime, the film thickness and deposited mass increment remain constant along with the deposition number. The linear growth mechanism is mostly reported for LbL assembly of strong polyelectrolytes or weak polyelectrolytes with a high charge density.[46–48] The film prepared accordingly is relatively thin, with high rigidity, low hydration level, and smooth surface. The exponential growth mechanism has mostly been observed on PEM systems comprising at least one weak polyelectrolyte.[49–53] In this growth mechanism, the film thickness and deposited mass per layer exhibit an increment following the deposition number increment. The exponential growth allows for the fabrication of thick PEM films (up to the micrometer domain) with a relatively small number of deposition cycles, with high surface roughness, hydration level, and softness.

There are different opinions regarding the mechanism behind the exponential growth pattern. The most widely accepted theories include the "in-and-out" diffusion model and the surface roughness model.[54–56] In the "in-and-out" diffusion model, at least one polyelectrolyte component diffuses fast "in and out" the entire film upon deposition, counting for the increment in film thickness and mass deposited over the film growth. [52,57] The Surface roughness model attributes the exponential growth to the rough nature of the film surface, which is claimed to lead to an increased surface area available for polyelectrolyte deposition, counting for the increment in the mass deposited following the deposition number.[55]

#### 1.2.3 Physical Parameters Affecting LbL Assembly

The physical condition of the LbL assembly has a significant impact on PEM film physicochemical properties. In the following section, I will briefly discuss the two most widely explored parameters, namely the solution pH and ionic strength of the polyelectrolyte solution.

**Solution pH** It is known that the charge density of a weak polyelectrolyte is strongly correlated to the pH value. As such, via adjusting the pH value, the charge density of a weak polyelectrolyte in an LbL assembly can be tuned. In particular, polyelectrolytes undergo a drastic charge density shift when pH is changed around the pKa value. For a weak polycation, the charge density is low at a pH value higher than its pKa. When the pH value decreases to close or even lower than the pKa value, the polymer undergoes a protonation process and acquires a high density of positive charges. A weak polyanion has a low charge density at pH values lower than the pKa and acquires a high density of negative charges through deprotonation when pH increases over the pKa.

The charge density of a polyelectrolyte is essential to the PEM film growth and the internal structure of the PEM film prepared correspondingly.[58,59] As a general trend, a high charge density favors the formation of a thin and rigid film; In contrast, a low charge density tends to result in a soft and thick film.[59,60] The possible effect of the charge density on the polyelectrolyte LbL process lies in two aspects. First, to overcompensate the surface charge, a higher adsorbed mass is required for polyelectrolytes with low charge density, leading to an overall higher film thickness. In this regard, by tuning the pH value, one can tune the relative charge density of the two polyelectrolytes and further tune the chemical composition of the multilayer. Second, the charge density has an impact on the conformation of the polyelectrolyte. High charge density leads to an extended and rigid conformation, while low charge density results in a more

coiled conformation. A high charge density and the extended conformation favors a high intrinsic compensation density and a rigid film, whereas a low charge density and the coiled conformation favors a low intrinsic compensation density and a soft film.[30]

Several studies have been focused on the effect of pH on the LbL assembly.[61–63] For instance, the study of Bieker et al. on PAA/PAH PEM has revealed a correlation of film growth patterns and properties upon pH variation (Figure 1.3).[61] At pH 6.5 to 7, both PAA and PAH are of a similar high charge density. The film is thin with a compact internal structure. When pH is moderately increased (8 to 10) or decreased (6.5 to 7), either PAA or PAH has a lower charge density, leading to a higher adsorption mass and a thicker film. When the pH further decreases (3 to 4.5) or increases (10 to 12), there is a great charge mismatch between the polyelectrolytes, which in turn decreases the LbL efficiency and the film thickness; In addition, there is a significant mismatch as to the adsorbed mass of each species at these domains. In my paper 1, I LbL assembled the P(PEGMEMA-*stat*-AMA) with alginate under pH 3, where the charge density of alginate is low. This low charge density facilitates the LbL assembly, providing a film with a wet thickness of a few hundred nanometers within 7 bilayers.



*Figure 1.3* Growth behavior of PAA/PAH PEM film, as a function of the assembly pH. Reprinted with permission from Ref [61]

*Ionic Strength* The ionic strength of the polyelectrolyte solutions can influence LbL efficiency, as well as film properties including the film thickness, morphology, surface roughness, and film permeability.[31,32,64] A few studies have demonstrated that, within a threshold, an increase in assembly salt concentration leads to an increase in film thickness of PEM films.[65,66] However, when the ionic strength is over a critical value, the film thickness starts to decrease until eventually no efficient LbL assembly is observed. (Figure 1.4a) In addition, Fery et al. have also demonstrated that an increased salt concentration can change the internal structure of the PEM film, inducing a pronounced softening to PAH/PSS multilayers.[67]



**Figure 1.4** Effect of ionic strength on PEM film properties: a) Thickness of a 20-layer PAA/PDADMA PEM film as a function of the salt concentration. Squares: PAA molecular weight 84 500, pH 11 deposition solution. Circles: PAA (MW 5200), pH 11. Triangles: PAA (MW 5200) from pH 5 solution (reprinted with permission from [68]); b) Schematic illustration of the transition from intrinsic to extrinsic compensations upon an increased salt concentration

The influence of ionic strength on LbL assembly can plausibly be attributed to two factors. First, the ionic strength has a significant impact on the polyelectrolyte chain conformation. At low ionic strength, the polymer chain tends to adopt an "extended rod" conformation, resulting in a thin and rigid PEM film; at high ionic strength, the polymer chain turns into a "globular coil" conformation, leading to a relatively thick and soft film. Second, the competition between extrinsic and intrinsic

compensations is the major cause of film thickness and property variation upon ionic strength change.[26,69] Particularly, the ions in the solution tend to break the intrinsic compensation and combine with the polyelectrolyte to form an extrinsic compensation instead (Figure 1.4b). Below a critical value, the increase of the ionic strength leads to a lower ionic cross-linking density in the film, giving rise to a thicker and less rigid film. Upon further increase of the ionic strength to a critical, the intrinsic compensation fails to hold the whole PEM film.

### 1.3 Synthetic Polyelectrolytes Designed for PEM Films

The physicochemical properties of a synthetic polyelectrolyte are essential to the functionalities of the PEM film prepared accordingly. Key parameters of a polyelectrolyte that control the behavior of the PEM film include chemical composition, copolymer structure, and charge density.[13] In this PhD work, these parameters were carefully evaluated to tailor the chemical structure and functionality of the PEM film. In this section, I will briefly discuss how these parameters affect the PEM film property, as well as the designing principle of the polyelectrolytes used in this work.

**Chemical composition** The chemical composition affects the properties of a polyelectrolyte in two aspects. First, the chemical structure of the ionizable repeating units determines some of the fundamental properties of a polyelectrolyte (polycation or polyanion, strong or weak polyelectrolyte). The most commonly reported ionizable groups include sulfonate group  $(-SO_3)$ . carboxyl group (-COO<sup>-</sup>), pyridine, and amino groups (-NH<sub>2</sub>, -NR<sub>3</sub><sup>+</sup>).[70] In this work, I selected AMA and MAA as the ionizable monomers. Primary amino and carboxyl groups were chosen due to their pH-responsiveness, which is essential for the preparation of pH-responsive thin films. In addition, primary amino and carboxyl groups are reactive to cross-linking agents such as glutaraldehyde and EDC/NHS. Hence, it is feasible to cross-link and stabilize the obtained thin film based on the corresponding chemistry. Second, as to copolymer-type polyelectrolytes, the chemical structure of the non-ionizable co-monomer can also affect the properties drastically. For instance, PNIPAM is well known as a thermos-responsive polymer showing LCST behavior in aqueous solution. PNIPAM-based polyelectrolytes have been synthesized and used in an LbL assembly process.[71] The incorporation of NIPAM introduced thermos-responsiveness to the PEM film prepared accordingly. In paper 1 and 2, I copolymerized PEGMEMA monomers with variable PEG side chain lengths with AMA and MAA and obtained P(PEGMEMA-stat-AMA) and P(PEGMEMA-stat-MAA) copolymers, respectively. The introduction of PEG side chains has

several impacts on the polyelectrolytes. First of all, PEG units bind with water molecules via hydrogen bonding in aqueous solutions, and incorporation of PEG units enhances the hydrophilicity of the polyelectrolyte, leading to a highly hydrated nature of the PEM films prepared accordingly. In addition, the PEG side chains provide a steric hindrance to the charges on the polyelectrolyte, resulting in a weakened polyelectrolyte complexation. Moreover, PEG units have been found to show protein repellence due to the high hydrophilicity. On this basis, in paper 2 I prepared a PEGMEMA-based polyelectrolyte film showing a resistance to BSA adsorption.

**Copolymer structure** Based on the structure, a copolymer can be categorized as a block, statistical, alternate, or graft copolymer. The most extensively studied copolymer-type polyelectrolytes used as a PEM building component are block and statistical copolymers. Here I will briefly discuss these two copolymer types. A block-copolymer polyelectrolyte comprises at least one block consisting of ionizable or charged repeating units. Block copolymer polyelectrolytes can be adsorbed on a surface via electrostatic interaction and have been used in surface lubrication, [72] stimuli-responsive surface preparation, [73] and so on. In terms of PEM films, self-organized micelles based on amphiphilic block copolymers have been used in the LbL assembly.[74–76] These films consist of multiple hydrophobic micelle core sites that are available for hydrophobic drug loading and controlled release.[77] In a statistical copolymer polyelectrolyte, the ionizable repeating units distribute on the polymer chain following a statistical rule. Specifically, a random copolymer is a special case of a statistical copolymer, where all the ionizable repeating units distribute randomly on the polymer chain. The random distribution of charges facilitates a homogeneous electrostatic adsorption of the polymer chain on the target surface. In addition, random copolymers are easily synthesized with a one-step copolymerization with a simultaneous feed of the co-monomers. The two random copolymer polyelectrolytes used in this work, P(PEGMEMA-stat-AMA) and P(PEGMEMA-stat-MAA), were synthesized adopting this approach. Notably, to ensure a random distribution of the ionizable monomer, both the non-charged and charged co-monomers adopt the same methacrylate backbone structure, and there is assumed negligible chemoselectivity between the co-monomers during the polymerization process.

**Charge density** The significance of charge density on LbL assembly has been discussed in section 1.2.3. In addition to the possible effect of charge density on the film thickness, chemical

composition, and internal structure, a critical minimum charge density has been reported, below which no effective LbL assembly can be achieved.[78,79] The charge density of a polyelectrolyte is determined by the number of ionizable groups and the degree of ionization of these groups. For a weak polyelectrolyte, the degree of ionization is dependent on the pH value. On the other hand, the number of ionizable groups can be tuned based on the design of the chemical composition of the polyelectrolyte. In terms of copolymer-type polyelectrolytes, the charge density can be tuned by adjusting the feed ratio of the charged and non-charged co-monomers in the polymerization. For the aim of this work, the ratio of the charged co-monomer was optimized to 25% for P(PEGMEMA-*stat*-AMA) and P(PEGMEMA-*stat*-MAA) copolymers. A too low charge density would fail to drive efficient film growth in the LbL assembly; a too-high charge density would lead to a low content of the PEGMEMA functional groups and compromise the functionality.

## 1.4 Polyelectrolyte Synthesis

All the polyelectrolytes used in this work were synthesized via ATRP. ATRP is one of the most widely explored controlled radical polymerization techniques since its development by Matyjaszewski et al. in the late 1990s.[80,81] There are two major advantages of adopting this technique. First, like all other controlled radical polymerizations, ATRP leads to a narrow molecular weight distribution. Second, the polymerization shares a "living" character, where the synthesized polymers can undergo further chain extension to construct complex polymer architectures such as block and graft copolymers. In this section, I will discuss the basic principle of ATRP. For the convenience of discussion, a brief introduction of free radical polymerization is first provided.

### 1.4.1 Free Radical Polymerization

Free radical polymerization has been long and widely employed in polymer synthesis. The polymerization is driven by the addition of a vinyl monomer onto the propagating polymer chain with an active free radical site. Three main stages are involved in free radical polymerization, namely initiation, propagation, and termination (Figure 1.5). In the initiation, free radicals are generated from an initiator molecule and transferred to a monomer, starting a reactive chain. Commonly used initiators include azo-compounds and peroxides, which can undergo hemolysis by heating to create free radicals. At the propagation stage, polymer chains grow with continuous monomer addition. In termination, the reactive radical sites on propagating chains are quenched

either by combination or disproportionation, ending the chain growth process. In addition to these three major stages, the propagating chains are also subject to chain transfer, where the reactive free radical site is destroyed and transferred to another component in the reaction mixture (solvent, initiator, monomer, polymer chain).



*Figure 1.5* Mechanism of free radical polymerization. I-I is the initiator; M is the monomer;  $P_m$  and  $P_n$  are propagating polymer chains; T is the chain transfer agent. Adapted with permission from [82]

#### 1.4.2 Atom Transfer Radical Polymerization

The radical concentration in a free radical polymerization is relatively high. As a result, the termination and chain transfer are significant and are the major sources of high polydispersity of the polymers (PDI ~2). Controlled radical polymerization was later developed to overcome these setbacks, the general principle of which involves a reversible deactivation process of free radicals. In this approach, the radical concentration is low, and the termination and chain transfer processes are drastically suppressed. As a result, a very narrow molecular weight distribution can be achieved, together with precise macromolecular structure control.

ATRP is one of the most widely used controlled radical polymerization techniques.[80,83,84] The basic mechanism of ATRP involves an equilibrium of propagating/dormant radical, predominantly in the form of an alkyl-halide/macromolecular species ( $P_n$ -X) and a transition metal (predominantly Cu(I)) catalyst (Figure 1.6).[84] On the one hand, the metal catalyst ( $Mt^m/L$ , m is the oxidation state and L denotes the ligand) can deprive  $P_n$ -X of the halide, activating (with a rate

constant  $k_{act}$ ) it into a free radical ( $P_n$ \*) that is reactive towards monomer propagation with a rate constant  $k_p$ . On the other hand, this process is reversible and the reactive propagating species is subject to deactivation (with a rate constant  $k_{deact}$ ) back to the dormant form. The activationdeactivation balance is predominantly favored towards the deactivation side. Therefore, a majority of the propagating chains are in the dormant status, resulting in a very low concentration of the reactive radicals in the system. Consequently, the termination and chain transfer processes are efficiently suppressed and the polymerization is controlled.



*Figure 1.6* Schematic illustration of the mechanism of ATRP. Reprinted with permission from [84]

Despite the significantly lower radical concentration, the termination cannot be eliminated and remains a major source of polydispersity. A precise control of the radical concentration and rate of polymerization is essential to achieving an optimal control of the polymerization. The rate of polymerization ( $R_p$ ) is described by the following equation:

$$R_p = k_p[M][P_n^*]$$
(1.1)

Where  $k_p$  is the polymerization rate constant,  $[P_n^*]$  is the radical concentration and is described as follows.

$$[P_n^*] = K_{ATRP} \frac{[P_n - X][Mt^m/L]}{[X - Mt^{m+1}/L]}$$
(1.1)

here  $K_{ATRP}$  is the ratio of the activation and deactivation rate constants,  $k_{act}/k_{deact}$ . According to this equation, the radical concentration can be controlled via two approaches. First, the concentration of the initiator ( $P_n$ -X), and metal catalyst ( $Mt^m/L$ ) can be adjusted. In addition, it is also plausible to drive the reaction equilibrium towards the deactivation side and reduce the radical concentration by adding the high oxidation state metal complex,  $X-Mt^{m+1}/L$ , upon triggering the polymerization. Second,  $K_{ATRP}$  is adjustable in order to tune the radical concentration.  $K_{ATRP}$  is determined by the

metal-halide bond strength in both  $P_n$ -X and X-Mt<sup>m+1</sup>/L. A higher  $K_{ATRP}$  value indicates a higher affinity of the metal catalyst towards the halide. Several key parameters affect the  $K_{ATRP}$  value, including the halide species, the ligand type, and the solvent. On the one hand, in terms of a Cu(I) catalytic system, the value  $K_{ATRP}$  of  $P_n$ -I is the lowest due to a very low iodophilicity of the Cu(I); The value  $K_{ATRP}$  of  $P_n$ -Br is the higher than that of  $P_n$ -Cl, due to a higher bond dissociation energy of  $P_n$ -Cl compared to  $P_n$ -Br. On the other hand, the ligand and solvent type has also a profound impact on the  $K_{ATRP}$  value (Figure 1.7). By a careful selection of the ligand and solvent, an optimal control over the polymerization can be achieved.



Low radical concentration

High radical concentration

**Figure 1.7** a) Effect of ligand on the value of  $K_{ATRP}$ , measured with ethyl 2-bromoisobutyrate as initiator, Cu(I) as the catalyst, acetonitrile as the solvent, at 22 °C. b) Effect of solvent on the value of  $K_{ATRP}$  value, measured with 2-bromoisobutyrates as initiator, Cu(I) as the catalyst, HMTETA as the ligand, at 25 °C. Reprinted with permission from [83]

ATRP also allows for an accurate estimation of the number-average degree of polymerization of the synthesized polymer, which is simply determined by the feed ratio of the monomer to the initiator, [M]/[Pn-X]. This method is based on the assumption of a 100% monomer conversion, which is not realistic. In reality, the monomer conversion can easily be estimated. One convenient

approach is to monitor the number of vinyl monomers by <sup>1</sup>H NMR with an internal standard. A detailed description of this approach is described in Section 2.1.2.

## 1.5 Physicochemical Properties of PEM Films

The functionality of a PEM film is strongly dependent on its physicochemical properties. The properties that govern the functionality of the PEM film can be categorized into two segments, namely the bulk properties of the film and the interfacial properties of the film. Commonly studied bulk properties include film mechanical property,[85,86] hydration level,[87,88] and pH-responsiveness.[89,90] They are essential in a series of applications such as controlled cell growth,[85] and delivery vehicles.[16] The interfacial properties of a PEM film are determined by the outer-surface chemical composition and the physical morphology such as surface roughness. The outer surface of the coating serves as a medium where direct interaction of the film with the ambience occurs. The outer-surface chemical composition and property dominate the performance of the films serving as hydrophilicity tuning[91,92] and selective adsorption.[93]

In this section, I will briefly summarize some relevant PEM properties related to this PhD work. In particular, I will focus on the pH-responsiveness, hydration, and interfacial property tuning of the PEM film.

### 1.5.1 pH-responsiveness

PEM films comprising weak polyelectrolytes are known to exhibit post-assembly pH-responsive behavior.[94–97] Upon pH shift from the assembly value, the PEM film follows a general trend of exhibiting a swelling conformation (Figure 1.8). This pH-responsive behavior is rationalized by the charge density dependence of weak polyelectrolytes on the pH value. Weak polyelectrolytes are protonated upon pH decrease and deprotonated upon pH increase over the pKa value. At the assembly pH, the obtained PEM is in an overall charge-balanced state. The overall number of positive and negative charges in the PEM film are roughly comparable. Upon pH variation, the PEM film exhibits an excess in positive/negative charge, leading to an amplified electrostatic repulsion and an increased osmotic pressure inside the film. Consequently, the film shows a swelling behavior. When the swelling exceeds a threshold, the internal polyelectrolyte complexation interaction fails to hold the whole film, and the film is subject to disintegration.



*Figure 1.8 pH-responsiveness of a (PAH/PSS) PEM microcapsules. The multilayers swell in alkaline conditions due to the unbalanced charges, reprinted with permission from ref* [97]

#### 1.5.1.1 Chemical Cross-linking to Enhance PEM Stability

As has been discussed in the previous section, weak polyelectrolyte based PEM films are subject to disintegration upon drastic pH variation. In order to stabilize the film and enhance its resistance to pH shifts, chemical cross-linking is frequently used. Depending on the cross-linkable functional groups, a range of different cross-linking reagents have been used such as carbodiimide cross-linkers, glutaraldehyde, genipin, and diazonium diphenylamine. In my work, I used two widely reported and highly efficient cross-linkers, glutaraldehyde and EDC/NHS. Hereafter I will introduce the principle and mechanism of these two cross-linking chemistries.

**Glutaraldehyde** has been widely studied and has found its use in cross-linking of thin films and biomaterials such as collagen and enzymes.[98–100] The success of the glutaraldehyde cross-linking lies in its low cost and hence high commercial availability, as well as its high reactivity.

The mechanism of glutaraldehyde cross-linking is described in Figure 1.9. Glutaraldehyde is a 5carbon dialdehyde, which binds rapidly to free amino groups utilizing the highly effective Schiff base chemistry. When introduced to the PEM film abundant in amino groups, both aldehyde groups in the molecule can combine with the amino groups from different polymer chains and form stable cross-links. Consequently, a network of polyelectrolyte forms in the PEM film, preventing the film from disintegration upon pH variation.



Figure 1.9 Schematic illustration of glutaraldehyde cross-linking mechanism

**EDC/NHS** is among the most readily available and widely used carbodiimide reagent for catalysis of amide formation from carboxyl and primary amine groups.[101] The history of exploiting EDC/NHS in PEM film cross-linking dates back to the early 2000s, when Richert et al. first cross-linked a PLL/HA PEM film and explored the corresponding effects on the improvement of stability and cell adhesion, as well as the elasticity property of the film.[102,103] Since then, EDC/NHS has become a popular agent in PEM cross-linking and has been used in various PEM films of weak polyelectrolytes comprising amino and carboxyl groups, including collagen/HA,[104] PAH/PAA,[105,106] PLL/PGA,[107] among others. EDC/NHS has demonstrated a range of advantages compared to other cross-linkers. As a water-soluble carbodiimide reagent, EDC can readily be used for cross-linking in aqueous solutions in the acidic-neutral pH range; after cross-linking, the residual EDC can be easily removed, with minimum compromise in biocompatibility; the EDC cross-linker only facilitates the amide formation, without incorporation of any cross-linker into the system.

The mechanism of the EDC/NHS coupling reaction is shown in Figure 1.10. Carboxylic acid is relatively inert to nucleophilic attack compared to its derivatives such as acyl halides and anhydrides. The activation agent, EDC, reacts with carboxylic acid groups, forming an O-acylisourea intermediate that is reactive to nucleophilic attack from primary amines in the film. The film is hence cross-linked by the correspondingly formed amide groups, releasing a urea by-product. The O-acylisourea intermediate is unstable in aqueous solutions. In parallel to the favorable amidation reaction, it might also undergo hydrolysis to regenerate the carboxyl acid, with an N-unsubstituted urea as the side product. To enhance the efficiency of the amide formation, NHS, or a more hydrophilic analog, sulfo-NHS, is often added to facilitate the EDC cross-linking reaction. NHS binds with the EDC-activated O-acylisourea intermediate, yielding a significantly

more stable NHS-ester, which allows for further conjugation to primary amines to obtain the target amide.[108]



*Figure 1.10* Schematic illustration of EDC/NHS Cross-linking mechanism. Adapted with permission from [109]

### 1.5.1.2 Tuning pH-responsiveness by Amine Quenching

In a typical cross-linking process with glutaraldehyde or EDC/NHS, the Schiff base or amide formation do not completely consume the free amine and carboxyl groups, and PEM films cross-linked accordingly are reported to exhibit pH-responsive swelling-shrinking behavior.[89,110] The excess free amine and carboxyl groups in the film remain responsive to protonation and deprotonation triggered by pH decrease and increase, respectively. As a result, the cross-linked film exhibits a cationic-zwitterionic-anionic transition along with the pH increase from acidic into the basic domain. Under the cationic and anionic states, the film adopts a swollen state; under the zwitterionic state, the film adopts a collapsed state.

Since the pH-responsiveness of such PEM films originates from the unconsumed amine and carboxyl groups, one approach to tune the pH-responsive behavior is to control the amount of free amine and carboxyl groups available. For example, in my paper II, I quenched the excess primary amine groups in cross-linked PEM films, to attenuate the pH-responsiveness in the acidic domain (Figure 1.11). To do so, the film was treated with EDC/NHS under the presence of a small molecular carboxylic acid, m-PEG3-COOH. The identical carbodiimide chemistry is used in this

process to combine free amine groups with the m-PEG3-COOH molecules in the solution. Consequently, free amine groups are quenched and substituted by the PEG groups consisting of three EG units.



1. EDC/NHS cross-linking; 2. Amine quenching with m-PEG3-COOH, EDC/NHS

**Figure 1.11** Schematic illustration of amine quenching with m-PEG3-COOH using EDC/NHS chemistry. Step 1: Amidation of carboxyl and amino groups catalyzed by EDC/NHS to form cross-links in the film; Step 2: Quench of excess amino groups to eliminate the remaining positive charges by amidation with m-PEG3-COOH catalyzed by EDC/NHS.

## 1.5.2 Hydration of PEM Films

The film hydration is a critical parameter of PEM film, affecting the film viscosity, permeability, and diffusion of functional components in the film.[111] Only a handful of papers can be found dealing with the hydration of a PEM film, probably due to the challenge in precise measurement of water content in the nanometer-range coating system. Some of the techniques used in determining the water content in a PEM film include neutron reflectometry,[39] Infrared spectroscopy,[112] and X-ray microscopy.[113] The combined QCM-D and optical ellipsometry technique has also been utilized in water content determination.[36,114] Specifically, the hydrated mass and dry mass of the film are obtained from QCM-D and ellipsometry measurements, respectively. The water content is calculated accordingly. In my work, I adopted a convenient approach of ellipsometry measurement for water content estimation according to the Bruggeman Effective Medium Approximation (BEMA).[115] This will be discussed in detail in Section 2.4.

The hydration of a PEM film is determined by a few factors. First, the hydration level is expected to show close relation to the ionic cross-linking density of the film. Generally, PEM films with high ionic cross-linking density demonstrate high rigidity and stiffness, with a low hydration level; PEM films with low ionic cross-linking density are soft, with a high hydration level.[30] Polyelectrolytes are highly hydrophilic due to the carrying charges and consequently are condensed by counterions and water molecules in aqueous solution. Over the LbL assembly process that forms the PEM, the polyion-counterion and polyion-water interactions are disrupted and the binding counterions and water molecules are released. Instead, the intrinsic compensation forms, cross-linking the multilayered film and leading to a dehydration process. For a film dominated by intrinsic compensation, the ionic cross-linking density is high, and the film is overall rigid and dehydrated; for a film dominated by extrinsic compensation, the ionic cross-linking density is low, and the film is overall soft and hydrated.[30]

The film's chemical composition is also crucial to the hydration of the film. On the one hand, the chemical nature of the ionizable repeating units affects the ionic cross-linking strength, and can further affect the ionic cross-linking density.[29,116,117] For instance, polycarboxylates are considered to be weak/labile polyelectrolyte complex former, and leads to weaker and hydrated PEM films, while sulfonates were claimed to yield strong and rigid polyelectrolyte complexes.[29] On the other hand, the non-charged part of a polyelectrolyte can also drastically affect the hydration of the PEM. For instance, PEM films comprising polyelectrolytes with a hydrophilic non-charged part, such as some polysaccharides, have been reported to exhibit very high water content of up to over 90%.[118–120] The high water content is attributed to the high hydrophilicity of the polysaccharides chemical structure, which easily forms hydrogen bonding with ambient water molecules.

In my work, I introduced another type of hydrophilic group, PEG, into the PEM film and obtained highly hydrated coatings accordingly. Specifically, I copolymerized PEGMEMA with a charged monomer (AMA) to prepare a highly hydrophilic polycation, P(PPEGMEMA-*stat*-AMA). Upon LbL assembly of the obtained PEGMEMA-based polycation with alginate, I prepared a PEM film with water content as high as over 90%. Additionally, via tuning the length of the hydrophilic PEG side chain, the water content of the PEM film is systematically tunable. This provides a novel approach to the fabrication of highly hydrated PEM film with tunable water content.

#### 1.5.3 The Interfacial Property of PEM films

PEM film interfacial properties are of high importance to the film functionality as the top layer serves as the direct medium for the interaction with the ambience. The chemical composition of the topmost layer of a PEM film has greatly affected the surface functionality including surface wettability, surface interaction with cells and microbial, fouling behavior of the surface, among others. Research effort has been put in tuning the interfacial properties of a PEM film. As an example, with careful tuning of the chemical composition of the topmost layer, superhydrophobic and superhydrophilic surface coatings based on PEM films can be prepared accordingly.[121] For instance, silver aggregates and gold clusters, among other hydrophobic materials, have been reported to be electrodeposited onto a PEM-based substrate layer to prepare superhydrophobic surface coatings. [92,122–124]

Conventionally the interfacial properties of a PEM film are determined by the last absorbed polyelectrolyte layer. To expand the scope of film applications, a common approach is to terminate the LBL process with a material which can provide the PEM film with a desired outer-surface functionality.[123,125–127] This approach is mostly adopted in the fabrication of the previously mentioned superhydrophobic or superhydrophilic surface coatings. Specifically, a PEM film is first assembled to create a thin-film substrate with a favorable nanostructure and surface architecture, followed by a deposition process with materials of desired hydrophilicity. Recently this technique has also been reported for some other applications. For instance, Li et al. deposited Ag nanowires on top of a bPEI/PAA–HA PEM and prepared a highly electrically conductive film with water-enabled healing ability.[128] In another example, Bai et al. coated a PEM film with carbon nanotubes and prepared a healable chemical gas sensor device.[125]

Another method to modify the PEM interfacial property is in-situ post-modification via chemical interactions. In this approach, the top layer of the PEM film is chemically modified, utilizing specific reactive functional groups in the layer and the corresponding chemistry. In one example, Li et al. post-modified a PEM coating with fluoalkylsilane, and successfully prepared a self-healing superhydrophobic surface coating.[129] In a more recent example, Delgado et al. prepared a PEM film comprising thiol groups in the top layer, and utilized the chemical reactivity of thiol groups to bind various functional materials on top of the coating.[93] With careful design and selection of the reactive functional groups in the outermost layer, this approach can demonstrate

exclusive advantages. For example, the modification process is versatile as to the types of the toplayer material and hence the surface functionality. In addition, by exclusive incorporation of functional groups in the outermost layer, the modification process shall not interfere with the bulk property of the whole film. Therefore, it is plausible to simultaneously tune the bulk property and the film interfacial property to obtain an optimized combination. In my paper 3, I adopted this approach and prepared a PEM film with a chemically tunable top layer. Specifically, a PAMA/PMAA PEM film functionalized with amino groups selectively in the outermost layer was prepared. These amino groups were then available for the modification of the outermost layer, without interfering with the inner-layer property such as pH-responsiveness.

# 2 Methods

## 2.1 Polymer Synthesis and Characterization

#### 2.1.1 Atom Transfer Radical Polymerization

ATRP was utilized for the synthesis of all the polyelectrolytes in this work. For the synthesis of **PEGMEMA-based copolymers**, the initiator (diethyl meso-2,5-dibromoadipate), catalyst (Cu(I)Cl) and ligand (Me6TREN) are dissolved in isopropanol at an optimized ratio of 1:1:2 to achieve the best control over the polymerization and the smallest PDI value of the obtained polymer. The theoretical DP was set to be 200, with the ratio of the charged co-monomers (BocAMA or tBMAA) set to be 25% (%DP). The reaction mixture was purged with argon to remove oxygen, after which the polymerization was triggered by immersing the reaction mixture to a 50°C water bath. After 16 h, the reaction is quenched by exposure to air, and the polymer is purified with dialysis against water. For the synthesis of the **homopolymers PAMA and PMAA**, the polymerization followed similar experimental details with different reagents and ratios. The ratio of the initiator (EBiB), catalyst (Cu(I)Cl), and ligand (PMDETA) was set to 1:0.5:1. The theoretical DP was set to be 100. Besides, Cu(II)Cl<sub>2</sub> was also added to decrease the radical concentration and achieve a better control of the polymerization. After polymerization, the reaction is quenched by exposure to air, and the polymer spurified with dialeve a better control of the polymerization. After polymerization, the reaction is quenched by exposure to air, and the polymer is purified by precipitation and filtration. All the polymers obtained are characterized by <sup>1</sup>H NMR spectroscopy and AF4.

Despite its versatility towards a wide range of materials, ATRP has been reported for setbacks in the presence of amine and carboxyl groups.[130–132] For amino groups, the Cu(I) catalyst is subject to a binding with the amine monomer and hence catalyst deactivation. For methacrylic acid, the Cu catalyst can be deactivated by coordination, and the *N*-containing ligands are subject to protonation by the acids. In order to eliminate the side reactions and guarantee an efficient polymerization process, a two-step protection-deprotection protocol was adopted in the syntheses involving both AMA and MAA. Specifically, for PAMA and its derivatives, a tBoc protected monomer, BocAMA, was utilized in the polymerization. The readily obtained polymer underwent a deprotection process with strong acid to obtain the desired amine-containing polycation.
Similarly, for the preparation of MAA involved polyanions, a t-butyl protected monomer, tBMAA, was used in polymerization, after which the butyl group is removed by TFA.

#### 2.1.2 Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy has been widely used in the characterization of chemical structure. A key factor of NMR spectroscopy is the spin of a nucleus, which is a quantum state of a particle defining its spin state. An atomic nucleus that has an overall spin of 1/2, such as <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F, is investigated in NMR spectroscopy. According to quantum mechanics, each of these nuclei has two possible spin directions corresponding to two magnetic quantum numbers: m = 1/2, and m = -1/2.[133] When exposed to an external magnetic field, the nuclei of the two spin directions are split into two different energy levels. (Figure 2.1) The energy gap is proportional to the applied magnetic field and is described by the Larmor frequency.



*Figure 2.1* Energy difference of nuclei of two different orientations, generated by the external magnetic field. Adapted from [133]

If the sample is exposed to an electromagnetic radiation of the Larmor frequency, the nuclei with m = 1/2 can absorb the radiation and jump into the higher energy state (m= -1/2), resulting in a strong absorption of the radiation.

The magnetic field at the nucleus is not equivalent to the external magnetic field. The electrons around the nucleus shield it from the applied magnetic field. Consequently, a chemical shift is

observed in the radiation spectrum, due to the difference in magnetic field strength created by the shielding effect of the electrons. The chemical shift is a function of the nucleus and the surrounding electron density, which depends on the chemical structure of the measured molecule. In this regard, the chemical structure of the compound can be elucidated. I have used <sup>1</sup>H NMR technique as a characterization method for the determination of polymer structures in my work. A Brucker 400 MHz NMR spectrometer was used, and TMS was used as the standard.

NMR was also utilized in my work as an important method to determine the DPs of the polymers obtained. To do so, a trace amount of an internal standard that does not interfere with the polymerization, DMF in this case, was added in the reaction mixture before the polymerization started. Samples of the reaction mixture were collected before and after the polymerization, and <sup>1</sup>H NMR spectra for both samples were measured. The integral ratios of monomer double bond (vinyl) / DMF standard before ( $r_1$ ) and after ( $r_2$ ) the polymerization were used to obtain the monomer conversion (c) via  $c = 1 - r_2/r_1$ . The theoretical number average molecular weight,  $M_n$ , was calculated accordingly using:

$$M_n = M_i + M_{mon}c \tag{2.1}$$

where  $M_i$  and  $M_{mon}$  are the molecular weights of the initiator and the monomer, respectively.

#### 2.1.3 Asymmetric Flow Field Flow Fractionation

The development of AF4 dates back to the 1960s when Giddings raised the concept of flow field fractionation.[134] Now, AF4 has become a state-of-the-art fractionation technique used for the characterization of macromolecules, nanoparticles, and proteins. Compared to SEC, the AF4 measurement demonstrates a few advantages. First, AF4 measures polymers with a broader range of molar mass than SEC. The fractionation remains efficient for polymer samples with a molar mass up to 10<sup>9</sup> g/mol. Second, the AF4 technique does not require a stationary phase used in the SEC. Therefore, the enthalpic interactions between the polymer samples (especially polymers containing polar functional groups such as polyelectrolytes) and the column packing is eliminated.[135] As a result, AF4 is of particular interest in characterizations of high-molecular-weight polymers, large-size nanoparticles, and polymers with a potential tendency of enthalpic interaction with SEC columns. AF4 is the major equipment characterization method in my work for the determination of molecular weight and polydispersity of all my synthesized polyelectrolytes.

The basic principle of AF4 is described in Figure 2.2. Instead of a packed column used in an SEC experiment, the fractionation in AF4 measurement occurs solely in a separation channel. The channel consists of two parts, the upper plate and the bottom plate. A spacer separates these two parts and creates a space where fractionation occurs. The bottom plate is made of a porous frit and is permeable, with a semipermeable membrane placed on top. The membrane is only penetrable for small molecules with a molecular weight below its MWCO. [136]



**Figure 2.2** Schematic illustration of separation mechanism in an AF4 measurement, where two compounds of different sizes are loaded;  $l_a$  and  $l_b$  are the centers of gravity of assembly of compounds A and B. Adapted from [136]

The fractionation of AF4 is achieved based on the diffusion coefficient differences of macromolecules with different sizes. There are two types of flows in the channel, a laminar flow in the axial direction, and a cross-flow in the perpendicular direction. [135] The laminar flow is a parabolic longitudinal flow, and the cross-flow is perpendicular to the membrane and creates a flow field. After the polymer sample is injected into the channel, two counteracting interactions are predominant on the perpendicular direction. On the one hand, the cross-flow drives the sample molecules to accumulation toward the membrane. On the other hand, the sample molecules undergo a counteracting diffusion and move away from the membrane by Brownian motion. A balance is eventually reached, where the injected compounds form a cloud of sample molecules with an equilibrium concentration distribution versus the distance against the membrane. The distance a compound can diffuse into the channel against the membrane ( $l_a$ ,  $l_b$  in Figure 2.2) is positively correlated to its diffusion coefficient. Large compounds are situated closer to the

membrane, while small compounds can diffuse farther away into the channel from the membrane. Under the parabolic laminar flow profile, the compound with a higher diffusion distance from the membrane moves faster (compound B); the compound with a lower diffusion distance moves slower (Compound A). The retention time  $t_R$  of a compound is depicted by the following equation: [135]

$$t_R = \frac{w^2}{6D} \ln\left(1 + \frac{V_x}{V_{OUT}}\right) \tag{2.2}$$

Where *w* is the channel width, and *D* is the diffusion coefficient of the sample molecule.  $V_x$  and  $V_{out}$  are the crossflow rate and detector flow rate, respectively.



*Figure 2.3* Schematic illustration of building components in the AF4 system

The AF4 system consists of a pumping system, an autosampler, a flow controller, a separation channel, a MALS detector, and an RI detector. The pumping system and the flow controller generate all the flows required in the separation channel and the system; The autosampler performs auto-injection of the sample into the system for measurement; The separation channel is the key component where the fractionation of the injected sample takes place; The system is equipped with two detectors, a MALS detector and a RI detector, for determining the molecular weight and the concentration of the fractionated sample, respectively. With the two detectors, a chromatogram with determined molar mass at each retention time is obtained, and the average molar mass and the polydispersity are calculated accordingly.

The MALS detector is used for determining the molecular weight of the polymer sample. The measurement is based on the principle of static light scattering, which is a frequently used

technique for measurement of weight average molecular weight and the radius of gyration of polymers. The setup of the MALS detector is shown in Figure 2.4. A high-intensity monochromatic light beam, normally a laser, is launched into the sample cell, where the light is scattered by the sample molecules at various angles. The intensity of the scattered light is dependent on the scattering angle, as well as the weight-average molar mass and radius of gyration of the sample molecule. In my work, the individual polymer chain has a relatively small size and fulfills the relation  $qR_g < 1$ . In this case, the intensity of the scattered light at a specific scattering angle is depicted by the Zimm equation[137]:

$$\frac{Kc}{R(\theta,c)} = \frac{1}{M_W} \left( 1 + \frac{q^2}{3} \langle R_g^2 \rangle \right) + 2A_2 c \tag{2.3}$$

Where  $M_W$  is the weight average molecular weight, c is the polymer concentration,  $R_g$  is the radius of gyration, and  $A_2$  is the second viral coefficient that describes the solubility of the polymer in the solvent.  $R(\theta, c)$  is the Rayleigh ratio, describing the scattering intensity of the sample molecule at angle  $\theta$ . The constant K is defined as:

$$K = \frac{4 \cdot \pi^2 \cdot \left(\frac{dn}{dc}\right)^2 \cdot 2 \cdot n_0^2}{N_A \cdot \lambda^4}$$
(2.4)

Where  $n_0$  is the refractive index of the solvent,  $\lambda$  is the wavelength of the incident light, and  $N_A$  is the Avogadro's number. The dn/dc is the refractive index increment of the sample macromolecule and can either be determined with the RI detector or obtained from tabulated values in literature. The scattering vector, q, is a function of the scattering angle  $\theta$ :

$$q = \frac{4\pi n_0}{\lambda} \sin \frac{\theta}{2} \tag{2.5}$$

In the online measurement, the fractionated sample solution in the MALS cell is highly dilute. Therefore, the term  $2A_{2}c$  is negligible and Equation 2.3 can be simplified as follows:

$$\frac{Kc}{R(\theta,c)} = \frac{1}{M_W} \left( 1 + \frac{q^2}{3} \langle R_g^2 \rangle \right)$$
(2.6)

According to Equation 2.6,  $Kc/R(\theta,c)$  obtained from various scattering angles is plotted versus  $q^2$ . The plot obtained accordingly is called the Debye plot with Zimm formalism, or the partial Zimm plot. [135] The  $M_W$  is obtained from the extrapolation of the curve to the zero angle (q = 0), and the  $R_g$  value is obtained from the slope of the curve.



Figure 2.4 Schematic illustration of the MALS detector in the AF4 system

**RI detector** The core component of the RI detector is a differential refractometer, which contains a flow cell with two parts: one sample cell and one reference cell. When performing the measurement, the reference cell is filled with the solvent, while the sample cell is for the sample solution. The differential refractive index (dRI) of the sample solution and the reference solvent is measured. The concentration of the sample solution is determined based on the dRI and the dn/dc value of the sample.

The RI detector is also used for dn/dc determination under the batch mode. To do so, a series of sample solutions with a concentration gradient is prepared and loaded sequentially into the sample cell. Accordingly, the dRI value of each concentration is obtained and plotted versus the concentration. The data are fitted linearly, and the slope of the linear fitting is the dn/dc value of the polymer sample measured. (Figure 2.5)



**Figure 2.5** Illustration of determination of refractive index increment (dn/dc) of P(PEGMEMA-stat-AMA) in pH 7,4 phosphate buffer at 633 nm and 25 °C. Five concentrations were used in the measurement: 0.05, 0.1, 0.25, 0.5, and 1 mg/mL. Plots of differential refractive index versus time (a) and differential refractive index versus concentration (b):  $dn/dc = 0.135 \pm 0.001 \text{ mL/g}$ 

#### 2.2 LbL Assembly

A range of technologies has been performed in LbL assembly, including immersive, spin, spray, electromagnetic, and fluidic assembly.[14] In my work, I used the fluidic technique for the fabrication of all the PEM films. All the PEM films are prepared in a QCM-D flow cell where the solutions are pumped into the cell with a peristaltic pump at a flow rate ranging from 75 to 150  $\mu$ /min. The concentration of polyelectrolyte solutions ranges from 100 to 300 ppm in the buffer with a selected pH value.

Figure 2.6 demonstrates the LbL process for PEM film fabrication. In order to graft the whole film onto the sensor after layer buildup, the sensor was pretreated with APTES to graft amino groups onto the silica substrate.[138] The substrate is hence positively charged and the polyanion is the first layer in the LbL assembly. The polyelectrolyte solutions are flowed into the QCM-D cell alternatively to achieve continuous layer buildup. Before switching from one polyelectrolyte solution to the other, a rinsing step with the buffer solution is applied to remove all the remaining polyelectrolyte solution in the cell from the previous adsorption.



Figure 2.6 Schematic illustration of PEM film fabrication by LbL assembly

#### 2.3 Quartz Crystal Microbalance with Dissipation Monitoring

Quartz crystal microbalance with dissipation monitoring (QCM-D) is a technique widely used in the study of thin films. It provides an estimation of the mass attached to the sensor, as well as its viscoelasticity behavior. The principle of QCM-D is based on the inverse piezoelectric effect of a quartz crystal, which exhibits cyclical deformation under an oscillating voltage. [139] In our QCM-D setup, an AT-cut quartz crystal sensor coated with a thin silica or gold layer as the substrate is used. (Figure 2.7a) The vibration of the quartz crystal follows a so-called "thickness-shear" model, where the two surfaces of the crystal vibrate in an antiparallel manner. (Figure 2.7b) When the wavelength in the crystal sensor reaches 2d/n, a resonance frequency  $f_n = nc/2d$  is reached, and a standing wave is created in the crystal sensor. [139] Here *d* is the thickness of the sensor, *c* is the speed of sound in the quartz crystal, and  $f_n$  is the resonance frequency of the quartz crystal sensor at the overtone number *n*. In our case, the resonance frequency of the sensor at the first overtone,  $f_{l}$ , is 5M Hz.



**Figure 2.7** Schematic illustration of QCM-D: (a) a 4.95 MHz AT-cut quartz crystal (Q-Sense). (b) Side view of the crystal. The two surfaces of the crystal vibrate cyclically in an antiparallel manner under oscillatory voltage; the first overtone (black waves at the edges of the crystal) and the third overtone (blue wave in the middle) are illustrated. Reprinted with permission from ref [139]

Two major parameters are recorded in the QCM-D measurement where polyelectrolytes are adsorbed to the sensor surface, namely the frequency, f, and the energy dissipation, D. On the one hand, a thin film deposited onto a quartz crystal sensor leads to a decrease in resonance frequency. The frequency shift ( $\Delta f$ ) hence provides information on the adsorbed mass. On the other hand, the external voltage is intermittently paused, and the energy dissipation (D) of the standing wave is recorded. The energy dissipation denotes the rate of decay of the oscillation and provides information on the viscoelasticity of the deposited film. [139]

Two models are utilized for the quantification of the QCM-D results, namely the Sauerbrey model, and the Voigt model.

**The Sauerbrey model** was developed by a German physicist, Gunther Sauerbrey, in 1959.[140] Based on this model, the normalized frequency shift for the *n*th overtone,  $\Delta f_n/n$ , and the areal mass density of the adsorbed film  $\Delta m$  follows a simple linear relationship:

$$\Delta m = -C \frac{\Delta f_n}{n} \tag{2.7}$$

Where C is a constant with a value of 17.7 ng cm<sup>-2</sup> Hz<sup>-1</sup> for the quartz crystal sensor with a fundamental resonance frequency of 5M Hz.

The Sauerbrey model is based on the assumption that the deposited film can be simplified as an extension of the quartz-crystal material. Therefore, it applies only to a system when a few criteria are met. First, the film thickness must be negligible compared to the thickness of the quartz crystal. Second, the deposited film must be rigid. Third, the film must be evenly distributed. Such films normally exhibit little deformation and damping factor. As a result, the energy dissipation shift  $\Delta D$  is relatively low. Generally, the ratio of the dissipation shift and normalized frequency shift,  $\Delta D/(-\Delta f_n/n)$ , shall be smaller than  $4 \times 10^{-7}$  Hz<sup>-1</sup>, for the Sauerbrey model to be valid.[139]

The Sauerbrey model fails to provide an accurate estimation of film mass and thickness when the adsorbed film is soft and viscoelastic. In this case, the film cannot be simplified as an extension of the quartz crystal, and the viscoelasticity shall be included in modeling. In this case, the **Voigt model** is used for the calculation of adsorbed mass and film thickness.[141] In this model, the deposited film is treated as a parallel combination of a spring and a dashpot. The former represents the elastic part, and the latter denotes the viscous deformation of the film. The complex shear modulus *G* is described as follows.

$$G = G' + iG'' = \mu_f + 2\pi i f \eta_f = \mu_f (1 + 2\pi i f \tau)$$
(2.8)

Where G' and G'' are the storage and loss modulus, respectively,  $\mu_f$  is the shear modulus of the film,  $\eta_f$  is the viscosity of the film, f is the oscillation frequency of the elastic spring, and  $\tau = \eta_f/\mu_f$  is the characterized relaxation time of the deposited film. The measured frequency and dissipation shifts can then be correlated to the film and medium properties by the following equations:[142]

$$\frac{\Delta f}{f} = -\frac{d_f \rho_f}{d_q \rho_q} \left( 1 - \eta_0 \rho_0 \times \frac{\left(\eta_f / \rho_f\right) \omega^2}{\left(\mu_f^2 + \omega^2 \eta_f^2\right)} \right)$$
(2.9)

$$\Delta D = \frac{d_f}{d_q \rho_q} \left( \eta_0 \rho_0 \times \frac{\mu_f \omega}{\mu_f^2 + \omega^2 \eta_f^2} \right)$$
(2.10)

where  $\omega$  is the angular frequency of oscillation,  $\eta_0$  and  $\rho_0$  are the ambience viscosity and density, respectively, and  $d_q$  and  $\rho_q$  are the thickness and density of the quartz crystal sensor, respectively. From the Voigt modeling the film thickness ( $d_f$ ), viscosity ( $\eta_f$ ), and shear modulus ( $\mu_f$ ) can be estimated.

#### 2.4 Spectroscopic Ellipsometry

In my PhD work, the LbL assembly process and pH-responsiveness tests of the films were

performed under simultaneous QCM-D and ellipsometry measurements. Spectroscopic ellipsometry is an optical technique used for the characterization of film thickness and optical properties.[143] In my work, it was performed *in-situ*, providing information on optical thickness, refractive index, and water content of the polymeric film along with the LbL assembly. Figure 2.8 shows a simplified illustration of the ellipsometry experimental setup.



*Figure 2.8* Schematic illustration of an ellipsometry experiment

The basic principle of ellipsometry involves the measurement of the change in polarization as light interacts with the flat sample layer.[143] The polarizer generates an elliptically polarized light that can be described as a combination of two components with orthogonal polarizations, namely the *p*-component and the *s*-component. The *p*- and *s*-components are parallel with and perpendicular to the plane of incidence, respectively. The incident light reflects and refracts at each of the interfaces when it interacts with the sample film, resulting in multiple light beams after reflection. These light beams can superimpose and interfere with each other. Consequently, the *p*- and *s*components undergo different decays in amplitude, leading to an overall change in the polarization state. The polarization change is commonly written in the form of the reflection coefficients ratio of *p*- and *s*-components,  $\rho = r_p/r_s$ , which is a complex function of the angle of incidence ( $\theta_0$ ), wavelength ( $\lambda$ ), optical functions of the substrate ( $n_s$ ) and the ambience ( $n_{amb}$ ), as well as the optical functions ( $n_j$ ,  $k_j$ ) and thickness ( $d_j$ ) of the film. Then, we can describe the ratio  $\rho$  by the following equation:[144]

$$\rho = \frac{r_P}{r_S} = \tan(\Psi) \exp(i \Delta) = \rho(\theta_0, \lambda, n_S, n_{amb}, n_j, k_j, d_j)$$
(2.11)

Here,  $\Psi$  and  $\Delta$  are defined as the amplitude ratio and the phase shift between the *p*- and *s*-polarizations, respectively. These parameters are measured as a function of the wavelength and the angle of incidence in an ellipsometry experiment.

An optical model is employed for modeling the data to obtain the film thickness and optical constant information. The model I used consists of three layers including a uniform hydrated polymer film, a silica coating, and a optically opaque titanium substrate. The silica coating and the titanium sbustrate were used as a description of the bare sensor. The corresponding layer thickness and optical constants were modeled before the polymer deposition and were used in the subsequent modeling for the polymer layer.

For a transparent film, the refractive index is often described by the Cauchy model:[145]

$$n(\lambda) = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4}$$
(2.12)

where *A*, *B*, and *C* are the fitting dispersion coefficients. In my work, *C* is often set to a fixed value of 0 to avoid over-parameterization, and only *A* and *B* are fitted.

The solvated polymeric film can also be considered as a two-component layer consisting of a dry polymer and water. In addition to film thickness, the volume fraction of water ( $f_w$ ) can also be estimated according to the Bruggeman Effective Medium Approximation (BEMA):[115]

$$0 = f_w \frac{n_w^2 - n^2}{n_w^2 + 2n^2} + (1 - f_w) \frac{n_p^2 - n^2}{n_p^2 + 2n^2}$$
(2.13)

where *n* is the refractive index of the hydrated film,  $f_w$  is the volume fraction of water, and  $n_w$  and  $n_p$  are the refractive indices of the water and dry polymer, respectively.

#### 2.5 Colloidal Probe Atomic Force Microscopy

Atomic force microscopy (AFM) is a scanning probe microscopy technique that is based on the interaction between a scanning mechanical cantilever and the sample surface. The first AFM was developed by Benning et al. in 1986.[146] It has since become a powerful technique in surface imaging, force measurement, and material manipulation at the atomic-level resolution.[147]

Figure 2.9 demonstrates the fundamental working principle of AFM. The AFM system I used (Nano Wizard 3, JPK Instruments AG, Berlin Germany) comprises four major components, a cantilever, a laser beam, a photodiode detector, and a piezoelectric scanner. In the experiment, the piezoelectric scanner drives the cantilever to scan over the sample surface. The cantilever is equipped with a tip that interacts with the sample surface, showing a deflection following Hook's law. The laser beam is focused on the back of the cantilever and is reflected in the photodiode detector. When the cantilever is deflected, a displacement of the laser spot on the photodiode detector is observed and used for the calculation of the interaction force between the tip and the surface.



*Figure 2.9* Schematic illustration of atomic force microscopy (Adapted from [148])

In this PhD work, I used the colloidal probe atomic force microscopy (CP-AFM) technique to study the interaction between a hydrophobic colloidal particle and my PEM film (See section 3.5). Instead of using a cantilever equipped with a sharp tip, I prepared a cantilever with a hydrophobic silica particle as the tip. To do so, a silica particle with a diameter of approximately 7 µm was first glued to a tipless cantilever. Afterward, the cantilever was hydrophobized by exposure to 50% undecyltrichlorosilane vacuumed desiccator with vapor in (v/v)а undecyltrichlorosilane/toluene solution. During the force measurement, the piezoelectric scanner drives the cantilever to approach and retract from the sample surface at a constant rate along the z-direction. (Figure 2.10a) The deflection of the cantilever z is calculated based on the displacement of the laser spot on the photodiode detector. Accordingly, the force between the colloidal probe and the PEM film surface is calculated by Hook's law:

$$F = kz \tag{2.14}$$

where k is the spring constant of the cantilever.

Figure 2.10b shows a schematic illustration of a force curve obtained from the measurement. Upon the approach of the cantilever from A to B, there is no force between the colloidal probe and the sample surface. The probe starts to interact with the surface at point B, where the probe jumps onto the surface due to an attraction force. Afterward, an increased repulsion force is observed upon further approaching. The interaction force turns from repulsion to adhesion upon retraction of the probe from the surface. A maximum adhesion force is reached at point C, after which the probe is detached from the surface and no further interaction is observed.



**Figure 2.10** Schematic illustration of CP-AFM: a) adhesion force measurement between the hydrophobic colloidal probe and the PEM film surface; b) Force curve obtained from CP-AFM measurement

# 3 Summary of Results

In this section, I discuss the major results and findings of my work. My work is dedicated to the fabrication of PEM films with tunable physicochemical properties and functionalities. To do so, I synthesized copolymers specially designed for the LbL assembly method and also conducted postassembly chemical modifications to tune the film properties. Specifically, my work can be divided into several aspects as follows. First, I designed and synthesized a range of random-copolymer type polyelectrolytes. For instance, PEGMEMA monomers were copolymerized with AMA and MAA, giving rise to a series of PEGMEMA-based copolymers bearing both types of charges. Second, using the tailor-made charged copolymers, I prepared PEM films incorporated with the desired functional component and tunable internal physicochemical properties. Specifically, I conducted LbL assembly the PEGMEMA-based polycations with alginate. By fine-tuning of the PEG sidechain length, I was able to tune the layer properties such as viscoelasticity and hydration level. Hereafter, I extended the scope of copolymer-type polyelectrolytes and prepared a singlecomponent PEM film by LbL assembly of PEGMEMA-based polycation and polyanion. Third, with post-assembly chemical modification, I managed to tune the PEM film properties such as pHresponsiveness and the outer-layer hydrophilicity. Finally, I aimed to construct a PEM film with tunable internal and interfacial properties and I investigated the adhesion between a hydrophobic colloid probe and the PEM films with different outer-layer hydrophilicity under different environmental conditions utilizing the CP-AFM technique. Unfortunately, this last part was only partially successful and will thus require modifications in the polymeric designs and new experiments before it can be summarized in a publication.

#### 3.1 Synthesis of Polyelectrolytes for PEM Fabrication

All the polyelectrolytes used in this work were synthesized by ATRP. To eliminate the deactivation of the Cu(I) catalyst by amino and carboxyl groups, a two-step protection-deprotection protocol was used. First, the AMA and MAA monomers were protected with Boc and t-Butyl groups, respectively, before they were used in ATRP to yield PBocAMA and PtBMAA polymers.

Thereafter, the polymers obtained were deprotected with TFA/DCM, to remove all the protection groups and yield the polyelectrolytes desired.

A summary of the polyelectrolytes synthesized in this work is shown in Figure 3.1. To start with, the homopolymers, PAMA and PMAA were synthesized. Specifically, a partially protected copolymer variation of PAMA, P(AMA-*co*-BocAMA), was obtained by partial deprotection of the PBocAMA. Furthermore, a series of copolymers were synthesized. PEGMEMA monomers were copolymerized with both AMA and MAA to obtain PEGMEMA-based polycations and polyanions, respectively; the HEMA monomer was also copolymerized with AMA to yield a P(HEMA-*stat*-AMA) copolymer.



# *Figure 3.1* Polycations (red) and polyanions (blue) synthesized and used in LbL assembly in this work

All the synthesized polyelectrolytes were characterized by <sup>1</sup>H NMR and AF4. <sup>1</sup>H NMR was used for the determination of the polymerization conversion and theoretical number average molecular

weight, M<sub>n</sub>. Also, it is a general method for polymer chemical structure characterization. AF4 was utilized for the determination of molecular weight and PDI value.

**PAMA, PMAA, PAMA-***co***-PBocAMA:** The synthesis of the homopolymers, PMAA and PAMA, follows a two-step protocol as discussed earlier. The protected polymers, PBocAMA and PtBMAA, were synthesized by conventional ATRP. The frequently used EBiB reagent was used as the initiator, and Cu(I)Cl/PMDETA was used as the catalyst. To achieve better control of the polymerization, Cu(II) was added to further reduce the radical concentration. The polymerization was conducted in an isopropanol solution at 50 °C. The reaction time was set to 18h, and a high conversion rate was confirmed by <sup>1</sup>H NMR spectroscopy, indicating a "living" nature of the polymerization. PBocAMA and PtBMAA were then deprotected with TFA/DCM. The complete disappearance of the characteristic t-butyl peak in the <sup>1</sup>H NMR spectrum indicated successful full deprotection. In order to obtain the partially deprotected PAMA-*co*-PBocAMA copolymer, the PBocAMA was treated with TFA/DCM for a relatively short time (10 min). The ratio of protected BocAMA was estimated by <sup>1</sup>H NMR to be approximately 63%. The parameters of the polyelectrolytes obtained were listed in Table 3.1. The PDI of the obtained polymers was relatively small (<1.5), suggesting a good control of the reaction.

Table 3.1	Properties	Properties of the synthesized PAMA, PMAA and P(AMA-co-BocAMA) polymer.									
		Mn¹ (kDa)	Mn² (kDa)	PDI <sup>2</sup>	tBocAMA content (DP%)						
PAMA		11.9	11.1	1.26	-						
PMAA		6.1	6.5	1.49	-						
РАМА-со-РВо	DCAMA	17.7	16.5	1.26	63%						

 Table 3.1
 Properties of the synthesized PAMA. PMAA and P(AMA-co-BocAMA) polymers

1. Determined with <sup>1</sup>H NMR; 2. Determined with AF4

**PEGMEMA-based Copolymers:** The PEGMEMA-based copolymers, P(PEGMEMA-*stat*-AMA) and P(PEGMEMA-*stat*-MAA), were obtained via copolymerization of PEGMEMA monomers with AMA and MAA, respectively. The copolymers were synthesized following a similar protection-deprotection protocol (Figure 3.2). A di-functional ATRP initiator, diethyl meso-2,5-dibromoadipate, which is widely used in symmetric multi-block copolymer construction[149–151], was utilized in this work, allowing for a potential chain extension to fabricate block copolymers with tailor-designed structures in future works. The reaction condition (solvent, temperature, reaction time) is the same as in the synthesis of PAMA and PMAA. The deprotection was also

confirmed by the complete disappearance of the Boc/tBu peak in <sup>1</sup>H NMR. Noteworthy, since both PEGMEMA and AMA/MAA have a methacrylate backbone, it can be assumed that the chemoselectivity between these two co-monomers can be neglected, and the obtained copolymer can be regarded as random. Nevertheless, here in this work I denote them more accurately as a statistical copolymer.



*Figure 3.2* Synthetic scheme for the P(PEGMEMA-stat-AMA) cationic copolymers. Reprinted from [110]

A summary of PEGMEMA-based copolymers synthesized in this work is provided in Table 3.2. Three P(PEGMEMA-*stat*-AMA) copolymers with different PEG side chain lengths were obtained (1-PPEGMEMA, m-PPEGMEMA, and s-PPEGMEMA). Here *l*, *m*, and *s* denote long, medium, and short, corresponding to the PEGMEMA monomers with average MW of 500, 300, and 211, respectively. Similarly, the P(PEGMEMA-*stat*-MAA) copolymer is denoted as m-PPEGMEMA(-). Here the symbol "-" denotes the negative charge for clarification. The content of amino/carboxyl groups in the copolymers was set to be 25%, which can be tuned by adjusting the feed ratio of the co-monomers.

The target DP of all four copolymers was set to be 200. A minor variation in DP was observed, due to a minor variation in conversion. As to the PDI, the incorporation of the PEGMEMA comonomer seems to yield a higher PDI than ATRP of AMA/MAA homopolymer. The PDI of s-PPEGMEMA is 1.43, which is relatively small. However, the PEG chain length increase led to an increased PDI, indicating a weakened control of the polymerization as the PEG side chain length increases. It might be due to a crosslinking effect between radicals and PEG units in the reaction mixture. Indeed, PEGMEMA copolymers have been reported to form cross-linked gels in free radical polymerization.[152,153] It was claimed that the cross-linking effect is a consequence of the relatively high chain transfer constants (of the order  $10^{-4} - 10^{-3}$ ) of oxyethylene units in PEG and PEG derivatives.

	M <sub>theo</sub> <sup>1</sup>	Mn <sup>2</sup>	00/2	Avarege MW of	Amine Content	Carboxyl Content	
	(kDa)	(kDa)	PDI⁺	PEGMEMA monomer	(D.P. %)	(D.P. %)	
I-PPEGMEMA	67.6	32.3	2.66	500	25	-	
m-PPEGMEMA(-)	32.5	29.0	1.74	300	-	25	
m-PPEGMEMA	46.7	34.5	1.71	300	25	-	
s-PPEGMEMA	36.9	38.2	1.43	211	25	-	

 Table 3.2
 Properties of the synthesized PEGMEMA-based copolymers

1. Determined by <sup>1</sup>H NMR; 2. Determined by AF4

**P(HEMA-stat-AMA)**: The P(HEMA-stat-AMA) copolymer comprising 75% HEMA repeating units was obtained with the same synthetic approach as PAMA and PMAA. The structure of the copolymer was confirmed by <sup>1</sup>H NMR. The theoretical  $M_n$  value was approximately 11 kDa. However, the AF4 measurement encountered an issue of drifting baseline in the RI signal, with no reliable chromatogram generated from the RI detector. The absolute molecular weight and PDI value remain to be determined.

#### 3.2 LbL Assembly of Alginate and PEGMEMA-based Copolymers (Paper 1)

In **Paper 1**, I prepared PEM films consisting of P(PEGMEMA-*stat*-AMA) cationic copolymers and alginate via LbL assembly. In terms of the polymer design of the P(PEGMEMA-*stat*-AMA) copolymers, I aimed for a constant charge density, degree of polymerization, and preferably polydispersity, but varying systematically the PEG side-chain length. In all, three P(PEGMEMA*stat*-AMA) copolymers with various PEG side-chain lengths, namely s-PPEGMEMA, m-PPEGMEMA, and I-PPEGMEMA, were LbL assembled with alginate. I first conducted the LbL assembly at various pH values ranging from pH 3 to 7. For pH values from 4 to 7, the PPEGMEMA depositions suffered a drastic detachment in the rinsing step. Significant layer depositions were only achieved under pH 3 regulated by a citric acid/Na<sub>2</sub>HPO<sub>4</sub> buffer. The layer buildup process was monitored in-situ with QCM-D. The frequency and dissipation shifts upon each deposition step were recorded (Figure 3.3a, b), indicating a successful layer buildup for all three PEM films.



**Figure 3.3** QCM-D frequency shift (a), dissipation shift (b), and the corresponding Voigt thickness (c) for LbL assembly of s-PEGMEMA (red square), m-PEGMEMA (blue circle) and l-PEGMEMA (yellow triangle) with alginate in pH 3 citric acid/phosphate buffer. Reprinted from Ref [110]

The simultaneous QCM-D and ellipsometry monitoring of the LbL assembly provided further insights regarding the film growth and physicochemical properties. First, the dissipation shifts provided information regarding film viscoelasticity. These PEM films comprising PEG units are highly viscoelastic, evidenced by high dissipation shifts; Moreover, the film viscoelasticity was observed to be directly correlated to the length of the PEG side-chain. This confirms that the incorporation of PEG units in the film can significantly increase the film viscoelasticity, which can be attributed to the high hydrophilicity of the PEG units. Second, the frequency shifts and estimated Voigt thicknesses provided insights regarding the growth mechanism. In general, two LbL growth mechanisms, linear and non-linear growth, have been reported in the literature. In my case, a difference in the LbL growth mechanism was observed for PPEGMEMA copolymers with different side-chain lengths. The s-PPEGMEMA/alginate layer deposition follows a non-linear trend, which is attributed to the diffusion of the s-PPEGMEMA chain "in and out" the PEM film. In contrast, the I-PPEGMEMA/alginate follows a linear growth pattern. This is speculated to result from the high steric bulkiness of the long PEG side-chain, which hinders segmental diffusion of the polymer chain into the film. Finally, the optical constants and hydration level of the film were obtained from the ellipsometry data using a single-component Cauchy and a two-component BEMA model, respectively. In general, the PPEGMEMA/alginate films were found to be highly

hydrated, showing a relatively high water content (>77%) and an optical constant close to water. (Figure 3.4) In addition, as the PEG side-chain length increases, the refractive index decreases, and the water content increases. To conclude, via incorporation of PEGMEMA units into a PEM film, it is feasible to obtain a film that is highly hydrated and viscoelastic. Moreover, by tuning the PEG side-chain length, one can finely tune the film growth mechanism, viscoelasticity, as well as the hydration level of the PEM film.



**Figure 3.4** Refractive indices obtained from ellipsometry modeling: 7 bilayers of s-PPEGMEMA/alginate (red), m-PPEGMEMA/alginate (blue), and l-PPEGMEMA/alginate (yellow) PEM films were modeled with single-component Cauchy (solid line) and dual-component BEMA (dashed line) models. Referential refractive indices for water (bold gray) and dry polymer (bold black) are provided. Reprinted from Ref [110]

After the LbL assembly, the PEM film was cross-linked with glutaraldehyde to enhance the stability against pH variation. Following that, the pH-responsive behavior of the cross-linked film was investigated by pH titration from 2 to 9 (Figure 3.5). The PEM film is responsive to pH variation because the charge densities of PPEGMEMA copolymer and alginate are both sensitive to pH. The PEM film is in a relatively collapsed state in the pH range of 3-4, due to charge neutrality within the film. At this state, the number of charged amino and carboxyl groups in the

film are comparable. Lowering the pH to 2 leads to protonation of amino and carboxyl groups in the film, thus creating a net positive charge. Consequently, counterions accumulate within the film, leading to an increase in osmotic pressure and hence swelling of the film. On the other hand, increasing pH to 9, results in the deprotonation of the amine and carboxyl groups, resulting in a net negative charge, followed by an osmotic swelling.



**Figure 3.5** *pH-responsiveness of the m-PPEGMEMA/alginate PEM film; (a) frequency and dissipation changes obtained from QCM-D; (b) Cauchy thickness (circle, dashed line), BEMA thickness (square, solid line) and water content (blue bar). The inset shows how the refractive index changes with the pH variation. Reprinted from Ref* [110]

### 3.3 Design of a Single-Component Cross-linked and Surface-grafted Polyelectrolyte Film Fabricated by an LbL Assembly Process (Paper 2)

In **Paper 2**, I prepared a single-component PEGMEMA-based polyelectrolyte film, which bears only one chemical component and one type of charge. To do so, the negatively charged m-PPEGMEMA- was LbL assembled with the positively charged m-PPEGMEMA copolymer to fabricate a PPEGMEMA-based PEM film consisting of seven bilayers in all. The LbL assembly process was monitored by QCM-D, and the results are shown in Figure 3.6.

Overall the LbL assembly of m-PPEGMEMA/m-PPEGMEMA- is more difficult than m-PPEGMEMA/alginate, probably due to a relatively low charge density and high steric hindrance resulted from the bulky PEG side chains. It is known that LbL assembly is greatly dependent on the deposition conditions including pH value, deposition time, and polymer concentration. These parameters were optimized to maximize layer adsorption. First, the pH values 6, 7, and 8 were probed, with little effect observed on film mass and thickness. The neutral pH 7 was eventually selected for layer deposition. Second, a longer deposition duration per layer was found to significantly decrease the layer desorption during the rinsing step. Hence, each layer was allowed to adsorb for 30 min, twice the duration of the m-PPEGMEMA/alginate LbL assembly. Third, an increased polymer concentration was found to decrease layer desorption over the rinsing step. Therefore, the polymer concentration was increased from 100 ppm used in m-PPEGMEMA/alginate LbL assembly to 300 ppm in this case.



*Figure 3.6* LbL of m-PPEGMEMA/m-PPEGMEMA- in pH 7 phosphate buffer monitored insitu by QCM-D: (a) frequency and dissipation shifts; (b) film thicknesses obtained by Voigt modeling. Reprinted from [154]

After successful layer buildup, the film was stabilized by chemical cross-linking. An EDC/NHS cross-linker was used due to several advantages. First, EDC/NHS is an efficient catalyst, facilitating the formation of an irreversible amide structure. Second, since EDC/NHS serves solely as a catalyst, no extra chemical structure is introduced in the film. Third, the catalyst is easily removed without residues in the film. After cross-linking, I performed a pH cycle test from pH 2.5 to 7 (acidic) and from pH 7 to 10 (basic), to investigate the pH-responsive behavior of the film. (Figure 3.7a) The cross-linked PEM film demonstrated a similar "swollen-collapsed-swollen" conformation change to the cross-linked PPEGMEMA/alginate film discussed in paper 1. At pH

7, the film is in a zwitterionic state and adopts a collapsed conformation; At pH 2.5, the film exhibits osmotic swelling due to the net positive charge; At pH 10, the film carries a net negative charge and exhibits osmotic swelling. This dual pH-responsive behavior of the film indicates the presence of both unreacted amino and carboxyl groups within the film.

In order to eliminate the amino groups and obtain a genuine "single-component" polyelectrolyte film with one chemical component and one type of charge, I quenched the remaining amino groups in the film with a small-molecular carboxylic acid, m-PEG3-COOH, with EDC/NHS. To do so, the PEM film was treated with a solution containing the quenching reagent (m-PEG3-COOH) and the carbodiimide catalyst (EDC/NHS) for 12h. The elimination of amino groups within the film was evidenced by the pH test. (Figure 3.7b) The responsive swelling of the film at pH 2.5 was significantly attenuated, while that at pH 10 remained unperturbed.



**Figure 3.7** Repeated pH cycles from 2.5 to 7, followed by 7 to 10: Frequency (blue circle) and dissipation (red square) shifts of m-PPEGMEMA/m-PPEGMEMA- PEM film for (a) a system cross-linked with EDC/NHS for 12 h (b) after amine quenching with m-PEG3-COOH. Reprinted from [154]

Finally, as a demonstration of the functional difference between the single-component polyelectrolyte film and the two-component zwitterionic film, I studied the protein repellence of the obtained PPEGMEMA polyelectrolyte film. To do so, a 5% (w/w %) BSA solution in pH 7.4 phosphate buffer was loaded into the QCM cell and the BSA adsorption was investigated. The zwitterionic film was found to significantly reduce the BSA repellence compared to the bare silica

substrate. This is attributed to the highly hydrated nature of the film comprising PEG units. However, the positive charges in the zwitterionic PEM are considered to have a negative impact on the BSA repellence. This is because BSA has an isoelectric point of approximately 4.7, and is effectively negatively charged at pH 7.4. Consequently, the removal of the amino group leads to a further reduction in BSA adsorption.



*Figure 3.8* Chemical structures of the polyelectrolytes and reagents used in paper 3, and the fabrication route for PMAA/PAMA PEM film with an aminated outer layer

#### 3.4 Tuning Interfacial Properties of PEM Films (Paper 3)

Interfacial chemical composition is important to the polyelectrolyte thin film properties including surface hydrophilicity and adhesion. Conventionally the interfacial property of a thin film assembled via LbL assembly is determined by the final layer adsorbed, and there lacks a systematic approach of chemically tuning the interfacial property. In **Paper 3**, I proposed a method to tune the interfacial property of a PEM film with minimum interruption on the internal structure. In particular, I prepared a PAMA/PMAA PEM film with amino groups situated selectively in the outermost layer. These amino groups are reactive towards chemical modification with various carboxylic acids using EDC/NHS cross-linking chemistry.

Before entering detailed discussions of each step of the film formation, I will briefly outline the fabrication procedure (Figure 3.8). **First**, a PAMA/PMAA PEM film was prepared. **Second**, a partially protected copolymer, PAMA-*co*-PBocAMA, was deposited as the final layer. As has been discussed in Section 3.1, PAMA-*co*-PBocAMA was prepared from a short-time deprotection of PBocAMA. Approximately 63% of the amino groups remain protected by Boc groups, estimated by the <sup>1</sup>H NMR spectroscopy. The amino groups in the polymer serve as the driving force of adsorption; the protected –NHBoc groups is to avoid consumption of these amino groups later in the amine quenching step. **Third**, the obtained film was stabilized by EDC/NHS cross-linking. Afterward, the free amino groups within the film were quenched using a similar approach as described in paper 2. By doing so most of the free amino groups within the bulk film were consumed. Consequently, in the next post modification step, the interruption to the internal structure of the film is minor, the interfacial property of the film is selectively tuned. **Finally**, the Boc protection groups in the outermost layer were removed in TFA/DCM and free primary amino groups reactive to chemical modification were created.

The successful LbL assembly process of PAMA/PMAA was confirmed by the in-situ monitoring of QCM-D (Figure 3.9a, d). Overall, 14 layers were deposited. The contact angles after deposition of layer 12 (PAMA) and 14 (PAMA-*co*-PBocAMA) were measured and a clear difference is observed (Figure 3.9b, c). A higher contact angle observed after the deposition of PAMA-*co*-PBocAMA is attributed to the higher hydrophobicity of the partially protected polymer. This confirms my hypothesis of having fewer amino groups on the partially protected copolymer and the successful final layer coating of PAMA-*co*-PBocAMA.

The QCM-D result also reveals the structural properties of the PEM film obtained. First, the dissipation shift over the layer deposition is small; Second, the film thicknesses modeled with Voigt and Sauerbrey models are comparable. Together these indicate that the PAMA/PMAA PEM film is rigid, with relatively a low hydration level, which can be attributed to a relatively large charge density of the polymers that can give rise to strong polyelectrolyte complexation as well as low monomer hydration. This thin and rigid nature of the film is the opposite of the highly hydrated and viscoelastic PPEGMEMA-based PEM film described in paper 1. The P(PEGMEMA-stat-AMA) copolymers used in paper 1 were designed with a much lower charge density and PEG units with high steric hindrance, leading to a weaker polyelectrolyte complexation inside the film. In

addition, PEG units are highly hydrophilic and combine a large number of water molecules in the PEM film consisting of PPEGMEMA copolymers. Whereas in PAMA/PMAA film, such a hydration effect is not facilitated. In summary, the PAMA/PMAA PEM film shows a strong polyelectrolyte complexation, low hydration level, and high rigidity. This, on the other hand, also demonstrates how the incorporation of PEG units into PEM films can drastically change the film's bulk property.



**Figure 3.9** LbL assembly of PAMA and PMAA, with PAMA-co-PBocAMA as the final layer at pH 4.4 citric acid/phosphate buffer: a) Frequency (blue) and dissipation (red) shifts throughout the LbL process, with odd layers of PMAA and even layers of PAMA (PBocAMA as the final layer); b) Voigt and Sauerbrey thickness growth along with PAMA/PMAA layer deposition; c) Contact angle measured at layer 12, with PAMA as the final layer; d) Contact angle measured at layer 12, with PAMA as the final layer in the final layer is provided to the process.



*Figure 3.10 pH-responsiveness of the film before (a) and after (b) amine quenching: frequency and dissipation shifts obtained form QCM-D during the pH cycle test (pH 4.5 and 2.5, pH 4.5 and 9); (c) Contact angle measured along the deprotection process, with a time interval of 30 min* 

After successful layer buildup, the PEM film was cross-linked with a similar method described in paper 2 with the EDC/NHS cross-linkers. Afterward, a pH cycle between pH 2.5 and 4.5 was performed and revealed the presence of free amino groups within the film (Figure 3.10a). These amino groups were then quenched with acetic acid in the presence of EDC/NHS as the catalyst. The successful quenching of amino groups within the film was confirmed by the repeated pH cycle test. Accordingly, an attenuation of film swelling at pH 2.5 was observed (Figure 3.10b). Finally, the Boc protection groups in the outermost layer were removed under TFA/DCM, giving rise to our target PEM film. The successful deprotection was confirmed by a decrease in the surface contact angle. (Figure 3.10c) This is rationalized by the detachment of hydrophobic Boc groups, and the exposure of hydrophilic amino groups in the outermost layer of the film.

As proof of concept, the deprotected PMAA/PAMA PEM film was tested for its outer layer modification. Two carboxylic acids with different chemical components, namely the undecanoic acid, and m-PEG3-COOH were used. These two surface modifying reagents were grafted to the

amine-functionalized surface by amide formation via EDC/NHS cross-linking (Figure 3.11). The surface contact angles after modification were measured and compared. There is an increase in contact angle for both films, indicating successful outer-surface modification. In addition, since undecanoic acid is more hydrophobic than m-PEG3-COOH, the PEM film modified by undecanoic acid showed a higher contact angle than that modified with PEG units.



*Figure 3.11* Contact angle measured before and after surface modification with undecanoic acid and m-PEG3-COOH with DCC as cross-linking agent

Finally, a pH cycle test was further performed on films modified by both undecanoic acid and m-PEG3-COOH, under the monitoring of QCM-D, to investigate the effect of surface modification on the internal structure of the film and its pH-responsive behavior (Figure 3.12). Both films exhibited a similar pH-responsive behavior as after amine quenching, indicating a minimum effect of the surface modification on the internal structure of the film. Also, despite a significant difference in top layer chemical composition and surface hydrophilicity, an overall similar pHresponsive pattern is observed for both films.



*Figure 3.12 Frequency and dissipation shifts corresponding to a pH cycle from pH 4.5 to pH 2.5, and pH 4.5 to pH 9, after surface modification with a) undecanoic acid, and b) m-PEG3-COOH* 

# 3.5 Interactions of PEM Films with Tunable Internal and Interfacial Properties (unpublished material)

I have demonstrated in Section 3.4 that the outer layer of a polyelectrolyte film can be modified for tuning the surface hydrophilicity without interfering with the internal structure and pHresponsive swelling behavior. In this section, I further investigated the interaction between a hydrophobic colloid probe and the PEM films showing different surface hydrophilicity using the CP-AFM technique. Three parameters were controlled and varied over the whole measurement, namely the outer layer chemical composition, the pH value, and the presence of a multivalent ion (Ca<sup>2+</sup>). The purpose of this study is to demonstrate how surface hydrophilicity and variations in the internal structure of the film affects the adhesion force and energy dissipation between the studied PEM film and a hydrophobic colloidal probe.

As the first step, I constructed a P(HEMA-*co*-AMA)/PMAA PEM film with PAMA-*co*-PBocAMA as the top layer (Figure 3.13). Here, a copolymer comprising 75% HEMA and 25% AMA was used as a substitution of the homopolymer PAMA. The purpose was to create a less dense and more hydrated layer by decreasing the number of intrinsic compensations as well as reducing the cross-linking density inside the film by reducing the content of the amino groups. The

film obtained was treated with the same chemical modification procedure described in Section 3.4. Two trends have been observed over the fabrication of the PEM film. First, the overall frequency shift was smaller than that of the PAMA/PMAA LbL assembly, while the energy dissipation shift of the film is larger. This opposite trend observed in QCM-D indicates the buildup of a film with less deposited mass and film thickness yet higher viscoelasticity. The lower adsorption mass is due to a significant desorption of the P(HEMA-*co*-AMA) polymer over the rinsing step, which is attributed to a lower charge density of the polymer and a weaker complexation; The higher viscoelasticity confirms a lower density of intrinsic compensation within the film. Second, the cross-linked PEM film, even without amine quenching, demonstrated much weaker pH-responsiveness to the acidic pH value (Figure 3.13d). It is speculated to be due to a much lower amine content in the film than the PAMA homopolymer. Nevertheless, the amine quenching was performed to minimize the amine content in the film. The outer surface of the film was then treated with the same approach to coat either m-PEG3-COOH or undecanoic acid as the outermost layer. The finally obtained PEM films demonstrate a similar pH-responsiveness behavior to that described in Section 3.4 (Figure 3.12).



**Figure 3.13** Properties of P(HEMA-co-AMA)/PMAA PEM film, with PAMA-co-PBocAMA as the final layer: (a) Frequency (blue) and dissipation (red) shifts from QCM-D, throughout the LbL process at pH 4.4 citric acid/phosphate buffer, with odd layers of PMAA and even layers of PAMA (PAMA-co-PBocAMA as the final layer); (b) Contact angle measured at layer 12, with PAMA as top layer; (c) Contact angle measured at layer 14, with PAMA-co-PBocAMA as the top layer; (d) pH cycle from 4.5 to 2.5, followed by 4.5 to 9, after film cross-linking with 5 mg/ml EDC/NHS: Frequency (blue) and dissipation (red) shifts obtained from QCM-D.

In the CP-AFM study, the variation of three parameters that affect the surface hydrophilicity and the internal structure of the PEM film were investigated, namely the outer layer chemical composition, the pH value, and the multivalent ion effect ( $Ca^{2+}$ ). The possible effects of these parameters on the adhesion force and energy form the key hypothesis of this study. In terms of the adhesion force, the PEM film modified with undecanoic acid shall demonstrate a stronger adhesion force than that modified with m-PEG3-COOH, due to a strong hydrophobic-hydrophobic interaction between the film outer layer and the hydrophobic colloidal probe. The adhesion energy is actually affected by both the surface hydrophobicity and the energy dissipation in the film bulk. First of all, to obtain high adhesion energy, the film is expected to have a hydrophobic outer layer with a strong adhesion force. In addition, the presence of  $Ca^{2+}$  is expected to have a positive effect on adhesion energy. The  $Ca^{2+}$  is known to form bridging complexes with the carboxylate groups, which serve as sacrificial bonds for energy dissipation and enhance the cohesion. As such, it was expected that the undecanoic acid-modified film in the presence of  $Ca^{2+}$  at pH 9 shows the highest adhesion energy. This hypothesis is illustrated in Figure 3.14.



*Figure 3.14* Schematic illustration of the adhesion force and energy between the hydrophobic colloidal probe and the PEM film: a) with the hydrophobic outer layer, in the presence of  $Ca^{2+}$  at *pH* 9; *b*) with the hydrophilic outer layer, at *pH* 4.5, without  $Ca^{2+}$ 

Table 3.4, Table 3.4, and Figure 3.15 presents the average adhesion force, average adhesion energy, and all the force curves measured under each condition, respectively. Concerning the effect of the surface hydrophobicity, the alkyl-terminated film showed generally a higher adhesion force,

confirming our hypothesis that the strong hydrophobic-hydrophobic interaction facilitates the adhesion. The effect of pH also confirmed the pH-responsive behavior observed in QCM-D in Section 3.4. Specifically, a similar adhesion observed at pH 2.5 and 4.5 indicates a similar hydration level and conformation; the decrease in adhesion observed at pH 9 is attributed to the swollen and hydrated nature of the PEM film. However, the effect of  $Ca^{2+}$  on enhancing the cohesion of the film and hence facilitating high adhesion energy was not observed. The adhesion force and energy of the undecanoic acid-modified film at pH 9 in the presence of  $Ca^{2+}$  are not significantly higher compared to other conditions.

The main cause of the low adhesion energy that contradicted our expectation is considered to be a weak adhesion force at a high pH, where the film adopted a highly hydrated state. Specifically, it is observed that when pH increased from 4.5 to 9, the alkyl-terminated film exhibited a significant decrease in the adhesion force. It is speculated that the grafted alkyl chains failed to offset the hydration of the film, leading to a decreased adhesion to the hydrophobic silica particle. That is, the outer surface was not hydrophobic enough to provide sufficient hydrophobic-hydrophobic interaction with the probe. As has been discussed earlier, high adhesion energy requires both a strong surface adhesion and a strong cohesion within the film. A significantly attenuated surface adhesion naturally resulted in low adhesion energy.

The relatively low hydrophobicity of the film is attributed to the low grafting density of the hydrophobic undecanoic acid. Since the density of the free amino groups in the PAMA-*co*-PBocAMA outer layer was only approximately 60%, the grafting density of the alkyl chain was probably not high enough to provide high hydrophobicity. Accordingly, to address this issue, a future step is to increase the grafting density by deposition of the two final layers with PAMA-*co*-PBocAMA. This increase in the number of PAMA-*co*-PBocAMA layers can increase the number of reactive amino groups in the outer layer, resulting potentially in a higher grafting density of the alkyl groups.

**Table 3.3**A summary of average adhesion force with standard deviation between the<br/>hydrophobic silica colloidal particle and the PEM films modified with undecanoic acid and m-<br/>PEG3-COOH

Adhesion force (pN)													
	PEG-terminated							Alkyl-terminated					
	NaCl			CaCl2			NaCl			CaCl2			
	2.5	4.5	9	2.5	4.5	9	2.5	4.5	9	2.5	4.5	9	
Average adhesion force	511	363	61	486	529	258	730	1173	148	706	727	375	
std	377	402	53	415	401	241	717	1140	222	585	666	384	

**Table 3.4**A summary of average adhesion energy with standard deviation between the<br/>hydrophobic silica colloidal particle and the PEM films modified with undecanoic acid and m-<br/>PEG3-COOH

Adhesion energy (J)														
	PEG-terminated							Alkyl-terminated						
	NaCl			CaCl2			NaCl			CaCl2				
	2.5	4.5	9	2.5	4.5	9	2.5	4.5	9	2.5	4.5	9		
Average adhesion energy	1.9 × 10 <sup>-18</sup>	1.4 × 10 <sup>-18</sup>	4.5 × 10 <sup>-19</sup>	2.0 × 10 <sup>-18</sup>	2.5 × 10 <sup>-18</sup>	1.1 × 10 <sup>-18</sup>	3.0 × 10 <sup>-18</sup>	7.1 × 10 <sup>-18</sup>	5.9 × 10 <sup>-19</sup>	3 × 10 <sup>-18</sup>	3.3 × 10 <sup>-18</sup>	1.8 × 10 <sup>-18</sup>		
std	2.4 × 10 <sup>-18</sup>	2.8 × 10 <sup>-18</sup>	9.4 × 10 <sup>-19</sup>	2.6 × 10 <sup>-18</sup>	3.4 × 10 <sup>-18</sup>	1.8 × 10 <sup>-18</sup>	6.0 × 10 <sup>-18</sup>	1.5 × 10 <sup>-17</sup>	1.3 × 10 <sup>-18</sup>	4.3 × 10 <sup>-18</sup>	5.1 × 10 <sup>-18</sup>	3.1 × 10 <sup>-18</sup>		



**Figure 3.15** Adhesion force curves for P(HEMA-co-AMA)/PMAA PEM films modified with m-PEG3-COOH and undecanoic acid. The adhesion force was measured in 100 mM NaCl adjusted to three pH values, pH 2.5, 4.5, and pH 9. The effect of Ca<sup>2+</sup> was examined by using a 5 mM CaCl<sub>2</sub> solution adjusted with NaCl to an equivalent ionic strength.

## 4 Outlook and Perspectives

Polyelectrolyte multilayers have been a powerful thin-film fabrication approach for surface modification. The functionalities of the surface are strongly correlated to the physicochemical properties of the PEM film. The primary focus of this PhD work is to tune the physicochemical properties of a PEM film via the design and synthesis of polyelectrolytes and the post-assembly modification. It has been demonstrated that, with careful control, the hydration level, pH-responsiveness, and the interfacial property of a PEM film can be tailored.

As a future direction, more functionalities can be introduced to PEM films adopting the approaches described in this work. For instance, it is plausible to copolymerize AMA and MAA with other functional monomers using the method described in Paper 1 and introduce novel functionalities. Also, the types of copolymers used in LbL assembly can be expanded to block and graft copolymers. As to the interfacial property tuning raised in Paper 3, a wider range of reagents can be used adopting the same approach. For instance, a range of bio-functionalized reagents can be grafted to tune the surface bio-adsorption, antimicrobial property, among others.

In addition, the concepts presented in individual studies in this work can be combined. For instance, the approach to tuning PEM interfacial properties raised in Paper 3 can be used in the PEGMEMAbased PEM films prepared in Paper 1. In this regard, it is feasible to simultaneously tune the bulk and the interface of a PEM film, achieving a precise control to the functionality of the coating.

Finally, this PhD work is mainly focused on the fundamental point of view, such as the methodology and the PEM physical chemistry. As the next step to this work, more emphasis can be on the application part. For instance, hydrated surface coatings reportedly demonstrate bio-antifouling and surface lubrication behaviors. The PEGMEMA-based PEM films prepared in Paper 1 are highly hydrated and can be explored for potential applications in this regard. Also, the PEM film with a modifiable outer surface can potentially be combined with membranes and serve as a separation technique.
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## Publications

## Paper 1

### PPEGMEMA-based Cationic Copolymers Designed for Layer-by-

### Layer Assembly



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## PPEGMEMA-based cationic copolymers designed for layer-by-layer assembly<sup>†</sup>

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We have synthesized three PPEGMEMA-based cationic copolymers with similar amine contents but with systematic variation in the average length of the PEG side chains. The positively charged copolymers were paired with alginate to fabricate layer-by-layer assembled multilayered films. It was demonstrated that the polymeric design, in terms of the systematic variation in the average length of the PEG units, affects the polyelectrolyte multilayer growth mechanism and can be used to tune the structural properties and the water content of the layers. In addition, by partial cross-linking of the amine groups present in the copolymer backbone, disintegration of the film induced by pH changes was prevented. Finally, it was demonstrated how the cross-linked multilayered film can exhibit cationic, zwitterionic and anionic properties depending on the pH value and how these changes are associated with swelling, layer contraction and changes in water content.

Received 16th July 2019 Accepted 22nd August 2019

DOI: 10.1039/c9ra05464b

rsc.li/rsc-advances

#### 1. Introduction

Layer-by-layer (LbL) assembly has drawn great attention as a convenient and adaptable method to fabricate functional polymer-coated surfaces.<sup>1–7</sup> The designated polymeric components are sequentially adsorbed onto a solid substrate, driven by different intermolecular forces, such as electrostatic attraction,<sup>2,3,8–10</sup> hydrogen bonding,<sup>11–14</sup> and covalent bonding.<sup>15,16</sup> The electrostatic LbL assembly method has been extensively investigated due to its simplicity and high efficiency.<sup>1</sup> Accordingly, polyelectrolyte multilayered films can be readily prepared by alternate immersion of a charged substrate into solutions of oppositely charged polyelectrolytes with rinsing steps in between. To this end, the thicknesses and composition of the layer are controlled by the number of deposited layers, pH,<sup>10,17,18</sup> ionic strength,<sup>10,19,20</sup> temperature,<sup>21</sup> adsorption time,<sup>22</sup> and polymer molecular weight.<sup>23</sup>

The need for ionizable groups in the backbone of the polymers for electrostatic LbL assembly effectively hinders the incorporation of uncharged polymers into the polyelectrolyte multilayered films. It is, however, feasible to accommodate desired functionalities into the backbone of charged polymers by chemical modification. Several authors have implemented such an approach to tune the functionality of multilayered films obtained by electrostatic LbL assembly.<sup>24–26</sup> For instance, Sun and coworkers modified polyethyleneimine (PEI) with polyethylene glycol (PEG) and fabricated a multilayered film based

on PEGylated-PEI and hyaluronic acid. The obtained film demonstrated antifouling properties resulting from the grafted PEG units.<sup>24</sup> In another relevant work, Neoh and coworkers custom synthesized poly(isobutylene-alt-maleic acid) (PIAMA) derivatives bearing either alkyl- or PEG-carboxyl side chains, and LbL assembled the obtained polyanions with PEI.25 It was shown that varying the side chains in the polyanion affects the wettability of the multilayered film; hence, such a film could be used to tune the protein adsorption and cell adhesion properties of the surface. In both the works mentioned above, the functional units (PEG or alkyl chains) are grafted onto a polyelectrolyte backbone, which has limitations in terms of tuning the properties of the polyion such as charge density and molecular architecture. A more versatile approach is to copolymerize neutral and charged monomers instead, where the former unit provides functional properties and the latter is responsible for the electrostatic interaction needed for LbL assembly. For instance, Schlenoff and coworkers copolymerized N-isopropylacrylamide (NIPAM, neutral monomer) with allylamine (cationic comonomer) as well as styrene sulfonic acid (anionic comonomer).27 The synthesized PNIPAM-based copolvmers were then paired to fabricate a thermoresponsive multilayered film. Based on this approach, one can thus control not only the chemical composition but also the charge density, molecular weight and chain architecture of the components in the multilayered film.

Poly(polyethylene glycol methyl ether methacrylate) (PPEG-MEMA) is a methacrylate derivative of polyethylene glycol that has been vastly studied as a potential coating material due to its hydrophilic nature and high water content, which leads to lubricating<sup>28,29</sup> and protein-repellent properties.<sup>30–33</sup> A common method to fabricate PPEGMEMA-coated surfaces is surface-

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<sup>†</sup> Electronic supplementary information (ESI) available. See DOI: 10.1039/c9ra05464b

initiated polymerization.<sup>34–37</sup> However, this method has several disadvantages that can restrict its applicability. It is generally challenging to achieve a thick coating using surface-initiated polymerization, which is a less versatile and scalable method that requires special substrate treatment and reaction control. In contrast, LbL assembly can be conducted through various methods, *e.g.*, dip-coating, spray-coating and spin-coating, which not only covers a broader range of substrates but can also render considerably thicker coatings.<sup>7</sup> Therefore, an alternative way to fabricate PEGMEMA-based coatings is to incorporate PEGMEMA units into charged chains so that electrostatic LbL assembly can be utilized.

Adopting this approach, we have synthesized a PPEGMEMAbased cationic copolymer in which PEGMEMA (neutral monomer) was randomly copolymerized with aminoethyl methacrylate (cationic comonomer). Three PPEGMEMA copolymer samples with comparable degrees of polymerization and amine content, but with different average lengths for the PEG side chains were prepared and then paired with alginate as a reference anionic polymer. This approach provided a method to study how systematic variation of the polymer architecture affects the multilayer buildup process and the multilayer structure. For this purpose, the assembly of the multilayered films was simultaneously monitored with a quartz crystal microbalance with dissipation monitoring (QCM-D) and spectroscopic ellipsometry. Accordingly, information for the thickness, conformation and water content of the films was extracted. Finally, the film structure was stabilized by crosslinking the amine groups, which preserved the pHresponsivity of the film while preventing its charge-induced disintegration.

#### 2. Experimental section

#### 2.1. Materials

Di(ethylene glycol) methyl ether methacrylate (DEGMEMA, 95%), poly(ethylene glycol) methyl ether methacrylate (PEG-MEMA) with number average molecular weights  $(M_n)$  of 300 and PEGMEMA with  $M_{\rm n}$  of 500 g mol<sup>-1</sup> were purchased from Sigma Aldrich. All three monomers were treated with neutral alumina column chromatography to remove the inhibitors before use. Sodium alginate (NaAlg, medium viscosity  $\geq 2.000$  cP, 2% (25) °C)), (2-Boc-amino)ethyl methacrylate (BocAMA, 99%), diethyl meso-2,5-dibromoadipate (98%), glutaraldehyde (50% aqueous solution), trifluoroacetic acid (TFA, 99%), (3-aminopropyl)triethoxysilane (APTES, 99%) and copper(1) chloride (CuCl, >99%, washed sequentially with acetic acid and ethanol before use) was purchased from Sigma Aldrich and used as received unless otherwise stated. Tris(2-dimethylaminoethyl)amine (Me6TREN, 99%) was purchased from Alfa Aesar, USA. All the solvents used in this work were obtained from Sigma Aldrich and of HPLC grade. A pH 3 citric acid/sodium phosphate buffer (1.62 g citric acid, 0.56 g sodium phosphate dibasic dehydrate in 500 mL water) was used for the preparation of the polymer solutions as well as for rinsing in between each deposition step. Solutions of 100 ppm (w/v) PPEGMEMA and alginate were prepared for LbL assembly and filtered through 0.44 µm regenerative cellulose

syringe filters to remove possible aggregates and dust. Ultrapure water (Milli-Q Plus system, resistivity of 18.2 M $\Omega$ -cm, TOC value less than 5 ppb.) was used to prepare all the solutions.

#### 2.2. Synthesis of poly(poly(ethylene glycol) methyl ether methacrylate)-*stat*-poly(aminoethyl methacrylate) (P(PEGMEMA-*stat*-AMA))

Atom transfer radical polymerization (ATRP) is a well-developed living radical polymerization (LRP) technique, which has been widely utilized in preparation of polymers with narrow molecular weight distribution and macromolecular engineering.38 PPEGMEMA copolymers with randomly distributed primary amine groups were synthesized by ATRP with a protectiondeprotection strategy according to previous reports.14,39,40 ATRP in the presence of primary amine groups (the cationic group) is challenging due to possible complex formation between the amine groups and Cu(1), which may readily inactivate the catalyst; thus, a two-step protocol was employed to overcome this problem. First, PEGMEMA was copolymerized with protected BocAMA, yielding a P(PEGMEMA-stat-BocAMA) statistical copolymer. In the second step, Boc groups were hydrolyzed under acidic condition (TFA/DCM), yielding the targeted P(PEGMEMA-stat-AMA) copolymer (Scheme 1).

The detailed ATRP experimental procedure is as follows. For the synthesis of the protected P(PEGMEMA-stat-BocAMA), diethyl meso-2,5-dibromoadipate (initiator, 7.2 mg, 0.02 mmol), PEGMEMA (monomer, 3 mmol), (2-Boc-amino)ethyl methacrylate (monomer, 229.27 mg, 1 mmol) and Me6TREN (ligand, 9.2 mg, 0.04 mmol) were dissolved in 5 mL isopropanol. Dimethylformamide (0.1 mL) was added to the reaction mixture as an internal standard for subsequent calculation of the monomer conversion. The reaction mixture was bubbled with nitrogen gas for 30 min, after which the prewashed copper(1) chloride (1.23 mg, 0.0125 mmol) was added. The polymerization was triggered by immersing the reaction flask into a 50 °C water bath. After 16 hours, the reaction was quenched by adding 5 mL of water into the solution and by exposure to air. A small volume of the solution (0.1 mL) was collected for <sup>1</sup>H NMR measurement to determine the reaction conversion. The polymer solution was then purified via dialysis (regenerative cellulose tubing with MWCO 6-8 kDa, Spectrum Laboratories, Inc.) against water for 3 days. Finally, residual water was removed via lyophilization to obtain the pure P(PEGMEMA-stat-BocAMA) copolymers. Fig. 1 presents the <sup>1</sup>H NMR spectra for one PPEGMEMA-based copolymer before and after the deprotection process. Accordingly, the peak corresponding to t-butyl (1.43) ppm) in the Boc protecting group is eliminated after the deprotection process.

In the deprotection process, the obtained P(PEGMEMA-*stat*-BocAMA) copolymers (300 mg) were dissolved in 4 mL dichloromethane followed by the addition of 0.5 mL of tri-fluoroacetic acid. The reaction mixture was stirred at room temperature for 3 hours, after which dichloromethane was removed by evaporation. The viscous polymer residue was redissolved in 5 mL of water, and the pH was adjusted to 7 with

Paper



1 M NaOH solution. The polymer was purified by dialysis and subsequent lyophilization, yielding a light-yellow viscous liquid.

All the P(PEGMEMA-*stat*-AMA) copolymers in this work were synthesized with the same procedure described above. In order to obtain polymers with a similar degree of polymerization (DP), the monomer to initiator ratio is fixed at 200 : 1 for all polymerizations, and the reaction time is set to be the same value of 16 hours to achieve high conversion (>80%) for all three polymerization processes. To determine the DPs of the polymers obtained, samples of the reaction mixture were collected before and after the polymerization process, and <sup>1</sup>H NMR spectra (Brucker 400 MHz NMR spectrometer) for both samples were taken using deuterated DMSO as the solvent. The integral ratios of  $\delta$  6.0 ppm (monomer double bond (vinyl))/ $\delta$  7.9 ppm (DMF) before ( $r_1$ ) and after ( $r_2$ ) the polymerization were used to obtain the monomer conversion (c) *via*  $c = 1 - r_2/r_1$ . The DP values were calculated accordingly and shown in Table 1.

The average number of ethylene oxide units in PEGMEMA monomers were tuned, in order to obtain P(PEGMEMA-*stat*-AMA) copolymers with different PEG side chain lengths. Overall

three P(PEGMEMA-stat-AMA) copolymers, namely P((EO)<sub>2.5</sub>-MEMA-stat-AMA), P((EO)<sub>4.5</sub>MEMA-stat-AMA) and P((EO)<sub>9</sub>-MEMA-stat-AMA), with average PEGMEMA monomer molecular weights of 211, 300 and 500, corresponding to an average 2.5, 4.5 and 9 ethylene oxide units in the PEG chains in each monomer were synthesized, respectively. To this end, PEG-MEMA with average molecular weights of  $M_{\rm n} = 300$  and  $M_{\rm n} =$ 500, were used for the synthesis of  $P((EO)_{4.5}MEMA-stat-AMA)$ and P((EO)<sub>9</sub>MEMA-stat-AMA), respectively, while a mixture of PEGMEMA with average molecular weights of  $M_n = 188$  and  $M_n$ = 300 in the ratio of 4:1 was used for the synthesis of P((EO)<sub>2.5</sub>MEMA-stat-AMA). For convenience, from now we will refer to these three copolymers as s-PPEGMEMA (for the shortest PEG units), m-PPEGMEMA (for the medium PEG units) and l-PPEGMEMA (for the longest PEG units), with s, m and l referring to short, medium and longer PEG side chains, respectively.

To determine the number average molecular weight  $(M_n)$ , weight average molecular weight  $(M_w)$ , and polydispersity index (PDI) of the copolymers, asymmetric flow field-flow



Fig. 1 NMR spectra for m-PPEGMEMA (a) after and (b) before deprotection to remove Boc groups.

#### Table 1 Properties of the synthesized P(PEGMEMA-stat-AMA) copolymers

	Polymer Compositi					
	P(EO) <sub>9</sub> MEMA	P(EO) <sub>4.5</sub> MEMA	DEGMEMA	AMA	$Mn^{b}$ (kDa)	$PDI^b$
l-PPEGMEMA	124	0	0	41	35.9	2.66
m-PPEGMEMA	0	135	0	45	38.3	1.71
s-PPEGMEMA	0	29	115	48	38.2	1.43
<sup>a</sup> determined by <sup>1</sup> H M	MR. <sup>b</sup> determined by AF	4.				

fractionation (AF4) was performed using a Wyatt Eclipse instrument with UV (Agilent 1230 infinity, Agilent), refractive index (Optilab rex, 633 nm, Wyatt) and multi-angle light scattering (MALS) (Dawn Heleos-II, 662 nm, Wyatt) detectors. A fritinlet channel equipped with a regenerated cellulose membrane (MWCO 5 kDa, Millipore) and a W350 spacer was used as the separation channel. The samples were analyzed with a constant detector flow of 0.5 mL min<sup>-1</sup>, and a cross flow that decreased exponentially from 3 mL min<sup>-1</sup> to 0 mL min<sup>-1</sup> in 20 min. A 50 mM PBS buffer at pH 7.4 was prepared and filtered with a 0.1 um membrane (Millipore) immediately before use as the eluent. The samples were dissolved in the PBS buffer with a concentration of 5 mg mL<sup>-1</sup>, and the injection volume was set to be 100  $\mu$ L. The refractive index increment (dn/dc) values for the copolymers were determined with an Optilab rex detector. For the molecular weight calculation, dn/dc values of 0.135, 0.120 and 0.118 were measured for s-PPEGMEMA, m-PPEGMEMA, and l-PPEGMEMA, respectively. Astra software (Wyatt, version 7.1.3.15) was utilized for data analysis and determination of the molecular weight from Debye plots.41

## 2.3. Quartz crystal microbalance with dissipation monitoring (QCM-D)

LbL assembly and the pH-responsiveness of PPEGMEMA/ alginate multilayered films was monitored with QCM-D (Q-Sense E1, Biolin Scientific, Gothenburg, Sweden) using silicacoated sensors (QSX 335, Biolin Scientific). In a typical QCM-D experiment, an alternating voltage is applied to a quartz crystal sensor, which gives rise to oscillation of the sensor at its fundamental resonance frequency (F). The resonance frequency is related to the mass of the oscillating sensor as well as any coupled mass. Additionally, the decay in the sensor oscillation is monitored upon repeated stopping of the drive generator output. Accordingly, the dissipation factor (D) is determined as the ratio of the dissipated energy to the total stored energy.

The sensor was first aminated with APTES before the LbL assembly.<sup>42</sup> To do so, the sensor was rinsed with copious amounts of ethanol and water, dried, and then plasma-treated (PDC-32G plasma cleaner, Harrick Plasma) in water vapor with a constant pressure of 0.5 Torr for 1 min. Afterwards, the sensor was exposed to APTES and toluene vapor for 18 hours by placing it in a vacuumed desiccator with a 50% (v/v) APTES/ Toluene solution. Hereafter, the sensor was rinsed with copious amounts of toluene and ethanol and dried with compressed air. The sensor was then immediately mounted

into the QCM-D module (QSense ellipsometry module, QELM 401, Biolin Scientific, Gothenburg, Sweden), and the measurement was started at 23 °C under a 150 µL min<sup>-1</sup> flow rate of citric acid/phosphate buffer with pH 3. After obtaining a stable baseline for all the harmonics, alginate and PEGMEMA solutions were alternately loaded and rinsed with buffer to obtain 7 bilayers in total. Glutaraldehyde solution (1%, w/w) was then loaded for 2 hours to cross-link the multilayered film, after which the chamber was rinsed with pH 3 buffer to remove the unbounded glutaraldehyde. To test the pH stability, 30 mM NaCl solutions with pH 3 and 5.6 were loaded, following which three consecutive pH cycles were conducted. Finally, the pHresponsiveness of the film was examined through titration of NaCl solutions with pH values ranging between 2 to 9. In all cases, the pH was adjusted with HCl/NaOH and NaCl was the only added electrolyte.

For thin, uniform and rigid films, the frequency shifts show a linear dependence to the adsorbed mass per unit area according to the Sauerbrey equation.<sup>43</sup> The Sauerbrey equation, however, only provides a valid estimation of the ratio if dissipation and normalized frequency shifts  $((\Delta D_n)/(-\Delta f_n/n))$  are significantly smaller than  $4 \times 10^{-7}$  Hz<sup>-1</sup>; otherwise, the adsorbed mass will be underestimated.44 For soft and highly hydrated polymeric films, as in the present case, the measured shifts (for different harmonics) in the resonance frequency and dissipation factor can be related to the thickness and viscoelastic properties of the film through modeling.44 Here, the viscoelastic Voigt model, where the adhered layer is represented by a film of uniform thickness and density with distinct viscous and elastic components can provide a better estimation of the film properties. The measured frequency and dissipation shifts are then related to the properties of the film and medium by:45

$$\begin{split} \frac{\Delta f}{f} &= -\frac{d_{\rm f}\rho_{\rm f}}{d_{\rm q}\rho_{\rm q}} \left( 1 - \eta_0\rho_0 \times \frac{(\eta_{\rm f}/\rho_{\rm f})\omega^2}{\left(\mu_{\rm f}^2 + \omega^2\eta_{\rm f}^2\right)} \right) \\ \Delta D &= \frac{d_{\rm f}}{d_{\rm q}\rho_{\rm q}} \left( \eta_0\rho_0 \times \frac{\mu_{\rm f}\omega}{\mu_{\rm f}^2 + \omega^2\eta_{\rm f}^2} \right) \end{split}$$

where  $\omega$  is the angular frequency of oscillation,  $\eta_0$  and  $\rho_0$  are the viscosity and density of the medium, respectively, and  $d_q$  and  $\rho_q$  are the thickness and density of the quartz crystal resonator, respectively. The density and viscosity of the medium together with the density of the hydrated film are estimated and treated

as fixed parameters to avoid overparameterization. The thickness  $(d_f)$ , viscosity  $(\eta_f)$  and shear modulus  $(\mu_f)$  of the polymeric film are thus obtained from Voigt modeling. The instrument software (Dfind, Biolin Scientific) was employed for analysis of the data. The density of the hydrated film  $(\rho_f)$  was estimated to be 1050 kg m<sup>-3</sup> (see ESI, Section S2†). The density and viscosity of water at 23 °C (from the software library) was used for the medium.

#### 2.4. Spectroscopic ellipsometry

As mentioned above, the QCM-D studies of the LbL assembly and tests of the pH responsiveness of the films were conducted in an ellipsometry module allowing for simultaneous QCM-D and ellipsometry measurement. Spectroscopic ellipsometry (M2000, JA Woollam Co., USA) is an optical method in which changes in the polarization state of light upon interaction with a sample are measured. Two parameters, *i.e.*,  $\psi$  (amplitude ratio) and  $\Delta$  (phase shift) are used to quantify the polarization change. The measured parameters correlate with the optical properties and thickness of the sample components:<sup>46</sup>

$$\tan(\psi)\exp(i\Delta) = \frac{r_{\rm p}}{r_{\rm s}} = \rho(\theta_0, \ \lambda, n_{\rm S}, n_{\rm amb}, n_{\rm j}, k_{\rm j}, d_{\rm j})$$

Here, the subscripts p and s refer to the parallel and perpendicular directions with respect to the plane of incidence, respectively, and  $r_p$  and  $r_s$  represent the reflection coefficients. The  $r_P$  to  $r_s$  ratio is a complex function of the angle of incidence  $(\theta_0)$ , wavelength  $(\lambda)$ , optical functions of the substrate  $(n_s)$  and the ambient  $(n_{amb})$ , as well as the optical functions  $(n_j, k_j)$  and thickness  $(d_i)$  of the film.

The QSense ellipsometry module (QELM 401, Biolin Scientific, Gothenburg, Sweden) allows in situ ellipsometry measurements on the QCM-D sensor through two optical windows. Accordingly,  $\psi$  and  $\Delta$  spectra were collected (wavelength range: 250–1000 nm, angle of incidence of 70°) before adsorption of the first layer (bare sensor data) and at the end of each rinsing step. The instrument software (CompleteEASE, JA Woollam Co., USA) was employed to model the  $\psi$  and  $\Delta$  data. The optical model employed was composed of three layers representing a uniform hydrated polymer film, a silica coating, and a thick optically opaque titanium substrate. First, the bare sensor was modeled as a pseudosubstrate, and the fitted parameters were fixed in the model (see ESI, Section S3<sup>†</sup>). Considering the low ionic strength of the buffer solutions, the optical data for pure water at 23 °C from the software library were used for the medium.47 The multilayered film was regarded as a transparent and homogeneous layer with no adsorption (k = 0); then, the refractive index (n) was described by the Cauchy equation:48

$$n(\lambda) = A + \frac{B}{\lambda^2}$$

In the first modeling approach, the hydrated multilayered film was considered as a single-component film characterized by a thickness and two effective optical constants ( $A_{\text{eff}}$ ,  $B_{\text{eff}}$ ). In

the second modeling approach, the multilayered film was considered as a two-component layer consisting of a dry polymer (A = 1.5 and B = 0.005)<sup>49,50</sup> and water. In addition to film thickness, the volume fraction of water ( $f_w$ ) was then estimated according to the Bruggeman Effective Medium Approximation (BEMA):<sup>51</sup>

$$0 = f_{\rm w} \frac{{n_{\rm w}}^2 - n^2}{{n_{\rm w}}^2 + 2n^2} + (1 - f_{\rm w}) \frac{{n_{\rm p}}^2 - n^2}{{n_{\rm p}}^2 + 2n^2}$$

where *n* is the refractive index of the hydrated film,  $f_w$  is the volume fraction of water, and  $n_w$  and  $n_p$  are the refractive indices of the water and dry polymer, respectively. Additional fitting options including surface roughness, grading, and thickness nonuniformity were tested, but only the latter was found to significantly improve the fitting quality. To assess the quality of modeling, the MSE value, uniqueness of the thickness, correlation between the fitted parameters and correctness of the refractive indices were checked (see ESI, Section S3<sup>†</sup>).

#### 3. Results and discussion

### 3.1. P(PEGMEMA-*stat*-AMA) cationic copolymers for multilayer assembly

As described in the experimental section, three PEGMEMAbased cationic copolymers, with varying average PEG side chain lengths were prepared by ATRP, as shown in Scheme 1; a summary of the properties of the synthesized copolymers is provided in Table 1. The conversion of each polymerization was determined from <sup>1</sup>H NMR data, based on which the actual degree of polymerization of each monomer was obtained. Overall, the degree of polymerization for all three copolymers was comparable, with a slight variation observed due to a minor difference in the conversion. The number average molecular weight and polydispersity of the copolymers was characterized by AF4 measurement (see Table 1). Here, it is seen that the s-PPEGMEMA has a relatively narrow molecular weight distribution, while the PDI value was observed to increase as the average PEG chain length increased. The latter observation indicates less control of the polymerization for longer PEG side chains.

#### 3.2. LbL assembly

Fig. 2 demonstrates the QCM-D data in terms of the shifts in the oscillation frequency (F) and dissipation factor (D) resulting from LbL assembly of PPEGMEMA/alginate multilayered films. The negative shift in oscillation frequency correlates to the effective mass (polymer film plus hydrodynamic water content) coupled to the sensor. The dissipation factor, on the other hand, represents the ability of the adhered film to dissipate the oscillatory energy; consequently, it is considered as a semi-quantitative measure of the film conformation and viscoelasticity. Accordingly, a soft and hydrated film is characterized by a relatively large positive shift in the dissipation factor, while a rigid and compact film produces relatively small shifts in the dissipation factor.

According to Fig. 2, the measured dissipation shifts for 14 layers (7 bilayers) for s-PPEGMEMA, m-PPEGMEMA and l-



Fig. 2 QCM-D frequency shift (a), dissipation shift (b) and the corresponding Voigt thickness (c) for LbL assembly of s-PEGMEMA(red square), m-PEGMEMA(blue circle) and l-PEGMEMA (yellow triangle) with alginate in pH 3 citric acid/phosphate buffer.

PPEGMEMA samples are approximately  $90 \times 10^{-6}$ ,  $180 \times 10^{-6}$ and  $183 \times 10^{-6}$ , respectively. Such large dissipation values suggest that the multilayered films are all swollen and highly hydrated. On the other hand, the frequency shifts for 14 layers for the s-PPEGMEMA, m-PPEGMEMA, and l-PPEGMEMA samples are around -1200, -1000 and -620 Hz, respectively. Accordingly, increasing the average length of the PEG side chains leads to a relatively larger dissipation shift (more viscoelastic film) and a relatively smaller frequency shift (smaller coupled mass). The model was then fitted to the frequency and dissipation data to estimate the Voigt thickness of each PPEGMEMA/alginate multilayered film as a function of the deposition number (see Fig. 2c and Table 2).

According to Fig. 2c, it appears that the LbL assembly of s-PPEGMEMA/alginate multilayered films follows a nonlinear growth mechanism. However, the multilayer growth seems to be inclined towards a linear growth mechanism when increasing the length of the PEG side chains in the copolymers. It has been suggested that the diffusion of polyelectrolyte chains "in and out" of the film during the LbL buildup can give rise to nonlinear growth.<sup>1,52–54</sup> Accordingly, one could speculate that the bulkiness of the PEG side chains can hinder segmental diffusion within the film, and, as a consequence, promote a linear growth mechanism.

In addition, while the frequency data show a monotonic gain, the dissipation data demonstrate a zigzag trend. Accordingly, adsorption of PPEGMEMA copolymers gives rise to a relatively large increment in dissipation, whereas the deposition of alginate produces a considerable decrement in dissipation. This can be interpreted based on the conformation of the interfacial top layer that has been previously observed.<sup>55</sup> When the PPEGMEMA copolymer is the top layer, a highly swollen and hydrated conformation is expected for the interfacial layer, which thus explains the large dissipation gain. On the other hand, adsorption of alginate seems to promote partial collapse of the sublayer, subsequently producing a relatively rigid and less hydrated interfacial layer.

Spectroscopic ellipsometry data can provide further information for the structural features of the multilayered films. Fig. 3 illustrates the  $\psi$  and  $\Delta$  spectra for the bare sensor as well as polymer-coated sensor after each deposition step. For s-PPEGMEMA, the LbL deposition is characterized by marked spectral oscillations in both the  $\psi$  and  $\Delta$  spectra. These changes are more pronounced specifically after the 10<sup>th</sup> deposited laver. where a secondary peak evolves in the UV range of the  $\psi$  spectra and a significant shift in the  $\Delta$  spectra is found. This observation is in line with the QCM-D data, suggesting a nonmonotonic growth in thickness. For m-PPEGMEMA, the changes in the  $\psi$  and  $\Delta$  spectra are relatively less pronounced, which can imply a relatively smaller thickness and (or) higher water content in the film. For l-PPEGMEMA, the evolution of the  $\psi$  and  $\Delta$  spectra is even less significant as no trace of a secondary peak is found in the  $\psi$  spectra and a relatively smaller shift in the  $\Delta$  spectra is demonstrated, which together suggests a thinner and (or) more hydrated layer compared to the layers obtained with s-PPEGMEMA and m-PPEGMEMA. In addition, the shifts in  $\psi$  and  $\Delta$  following LbL deposition indicate a monotonic trend, which suggests a linear growth mechanism.

Table 2	Summary of the	QCM-D and ellipsome	ry data obtained for the	PPEGMEMA/alginate r	nultilayered film
	2		2	<u> </u>	5

polymer	Voigt Thickness (nm)	Cauchy Thickness (nm)	BEMA Thickness <sup>a</sup> (nm)	BEMA Water Content <sup>a</sup> (%)	Dry Thickness <sup>b</sup> (nm)
s-PPEGMEMA	225.1	$200.7\pm0.3$	$198.9\pm0.3$	$77.3 \pm 0.1$	$48.9\pm0.1$
m-PPEGMEMA	273.8	$180.6\pm0.6$	$177.1\pm0.6$	$83.3\pm0.1$	$29.4\pm0.1$
l-PPEGMEMA	297.6	$163.3\pm2.7$	$156.1\pm2.6$	$91.5\pm0.2$	$17.6\pm0.1$

<sup>a</sup> Fitted with the BEMA method, Bruggeman model. <sup>b</sup> Fitted with the Cauchy method, with mean A, B and C parameters for PPEGMEMA and alginate Cauchy parameters.

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Fig. 3 Ellipsometric Psi (top row) and Delta (bottom row) for LbL assembly of s-PPEGMEMA (a and b), m-PPEGMEMA (c and d) and I-PPEGMEMA (e and f) with alginate in pH 3 citric acid/phosphate buffer (the black solid curve represents the bare sensor and the arrows hint at LbL deposition).

To obtain more quantitative information for the structure of the films, the spectroscopic ellipsometry data were examined using a single-component Cauchy and a two-component BEMA model. According to both modeling approaches, increasing the average length of the PEG side chains leads to a decrement in the total hydrated film thickness from approximately 200 nm for the s-PPEGMEMA/alginate multilayered film to approximately 160 nm for the l-PPEGMEMA/alginate multilayered film, while the water content within the film increases roughly from 77% for the s-PPEGMEMA/alginate multilayered film to 91% for the l-PPEGMEMA/alginate multilayered film. This difference in the water content of the films is reflected in the refractive index dispersion of the hydrated layers (see Fig. 4). As a general observation, the refractive indices for all three layers are closer to that of water than of the dry polymer, suggesting a large amount of water in the films. In addition, increasing the average PEG side chain length gives rise to a decrement in the refractive index, suggesting that the water content is relatively large. The increase in water content obtained upon increasing the average PEG side chain length can also explain the larger QCM-D dissipation shift for this layer. In addition, the dry thicknesses of the s-PPEGMEMA, m-PPEGMEMA, and l-PPEGMEMA samples are found to be approximately 49, 29 and 18 nm, respectively. Accordingly, it can be concluded that increasing the average length of the PEG side chains decreases the amount of adsorbed polymer chains, which can be attributed to a larger steric hindrance for LbL deposition.

It is noteworthy that a discrepancy in film thickness was observed between Voigt modeling and the optical thickness (Table 2). We attribute this discrepancy to the inhomogeneous nature of the PPEGMEMA/alginate multilayered film. In particular, while the inner layers of the obtained film are relatively impact, the outer PPEGMEMA layer is highly swollen and hydrated. The sparsely distributed PPEGMEMA chains in the outermost layer of the film significantly affect the dissipation value and add to the overestimation of the Voigt thickness. This



**Fig. 4** Refractive indices obtained from single-component Cauchy (solid line) and two-component BEMA (dashed line) models for 7 bilayers of s-PPEGMEMA/alginate (red), m-PPEGMEMA/alginate (blue) and l-PPEGMEMA/alginate (yellow) multilayered films. Refractive indices for water (bold gray) and dry polymer (bold black) are provided for comparison.

effect is much less significant to ellipsometry modeling, where an outer layer with low polymer density will contribute with a low weight to the optical thickness.

#### 3.3. Stabilization of the PPEGMEMA/alginate film by crosslinking

As electrostatic interactions are the driving force for the multilayer buildup, the pH at which the multilayered film is fabricated should roughly correspond to a state of charge neutrality. Accordingly, a subsequent change in pH will create a charge imbalance that will cause excessive swelling and disintegration of the film (see ESI, Fig. S17†). Chemical cross-linking, on the other hand, is an effective method to enhance the pH-stability of polyelectrolyte multilayered films. Glutaraldehyde, as a common cross-linker for proteins and polysaccharides, can form a dynamic covalent bond between free amine groups on the PPEGMEMA copolymer through the Schiff base reaction.<sup>56–58</sup> Herein, we selected the m-PPEGMEMA/alginate multilayered film to investigate the effect of cross-linking and pH-stability.

Fig. 5 demonstrates the structural changes in the multilayered film resulting from the cross-linking process. With respect to the QCM-D data (Fig. 5a), a significant decrement in the dissipation of around  $-80 \times 10^{-6}$  accompanied by a small frequency shift of around -70 Hz, is observed. While the change in frequency can be considered insignificant compared to the total frequency shift during the layer buildup (see Fig. 2a), the relatively large decrease in dissipation suggests structural collapse and enhanced rigidity of the film after cross-linking.

According to the ellipsometry data, chemical cross-linking is accompanied by a decrease in the optical thickness from 237 nm to 210 nm, confirming the film shrinkage, as inferred by the dissipation data. In the same line, a decrease in water content from 85% to 81% is demonstrated. The film shrinkage could be attributed to the consumption of a portion of the amine groups, and, hence, charge regulation within the film, as well as the conformational immobilization caused by the chemical cross-links.

## 3.4. pH-responsive behavior of the m-PPEGMEMA/alginate multilayered film

Fig. 6 demonstrates the stability of the multilayered film upon three consecutive pH cycles between 3 and 5.6. According to the QCM-D data (Fig. 6a), increasing the pH from 3 to 5.6 leads to a gain in dissipation and a decline in frequency, which both imply swelling and hydration of the polymer film. By lowering the pH back to 3, the frequency and dissipation shifts demonstrate conformational collapse and dehydration of the film. During the three pH cycles, no significant layer disintegration and mass loss was observed, indicating enhanced pH stability of the film after cross-linking. It is, however, worth mentioning that a slight structural hysteresis (irreversible frequency and dissipation shifts) is found after each pH cycle, which could be attributed to conformational restructuring within the film that can also cause irreversible ionization of amine and carboxyl groups.<sup>59–61</sup>



**Fig. 5** (a) Frequency (blue) and dissipation (red) shifts for the crosslinking step of a m-PPEGMEMA/alginate multilayered film with 1% glutaraldehyde; (b) Psi (blue) and Delta (red) before (dashed line) and after (solid line) cross-linking. The inset shows the modeling result obtained from the Cauchy and BEMA methods.

Ellipsometry results (Fig. 6b) represent the same effect as concluded from the QCM-D data. During pH cycling, the Cauchy thickness exhibited a zig-zag trend in agreement with the frequency and dissipation shifts. Accordingly, the optical thickness obtained with the BEMA model fluctuates between approximately 210 nm at pH 3 and 225 nm at pH 6, while the estimated water content at the same time also varies systematically.

To further investigate the pH-responsiveness of the PPEGMEMA/alginate multilayered film, a pH titration was performed (see Fig. 7). For the QCM-D data presented in Fig. 7a, it can be seen that increasing the pH from 2 to 4 results in a relatively large dissipation shift of around  $-50 \times 10^{-6}$ , suggesting shrinkage of the film. In contrast, further increasing the pH from 4 to 9 is characterized by a relatively large gain in the dissipation of approximately  $140 \times 10^{-6}$ , indicating



**Fig. 6** m-PPEGMEMA/alginate multilayered film stability as a function of pH during repeated changes between pH 3 and 5.6: (a) frequency and dissipation changes monitored with QCM-D; (b) Cauchy (dashed line, circle) and BEMA thicknesses (solid line, square) as well as water content (violet bar) obtained *via* the BEMA model from ellipsometry.

a reswelling of the film. For the frequency shift, a similar variation is observed although the trend is less clear.

In the ellipsometry data (see Fig. 7b), increasing the pH from 2 to 4 is associated with a decline in the BEMA thickness from 235 nm to 208 nm, while further increasing the pH results in a gain in thickness from 208 to 290 nm. The estimated water content followed the same trend as the film thickness, *i.e.*, decreasing from 87 to 85% when the pH is changed from 2 to 4 and increasing from 85 to 89% when the pH is changed from 4 to 9.

The swollen-collapsed-swollen conformational change of the multilayered film when the pH is first changed from 2 to 4 and then subsequently changed to 9 can be explained based on pH-dependent charge regulation within the multilayered film. The collapsed state in the pH range of 3–4 could be attributed to the charge neutral state within the multilayered film. Under such conditions, the number of charged amine and carboxyl groups within the film are roughly comparable. Decreasing the pH to 2 is associated with protonation of the amine groups on the



**Fig. 7** pH responsiveness of a m-PPEGMEMA/alginate multilayered film; (a) frequency and dissipation changes obtained from QCM-D; (b) Cauchy thickness (circle, dashed line), BEMA thickness (square, solid line) and water content (blue bar). The inset shows how the refractive index changes with the pH variation).

PPEGMEMA copolymer and the carboxylate groups on the alginate, creating a net positive charge within the film. This leads to the accumulation of counterions (Cl<sup>-</sup>) within the film that can produce an osmotic pressure difference, and, hence, swelling of the film. On the other hand, increasing the pH to 9 is accompanied by deprotonation of the carboxyl groups and amine groups, which produces a net negative charge in the film. Accumulation of Na<sup>+</sup> counterions then results in an osmotic swelling. Thus, the swollen-collapsed-swollen conformational change is due to the cross-linked PPEGMEMA/alginate multilayered film exhibiting cationic, zwitterionic and anionic states as a function of pH. To this end, we note that the charging state of the multilayered film, as well as the possibility to change between different charging states, is of high importance for the film functionality and for possible applications. While both the hydration level and the sign of the surface potential will be decisive for the films ability to either attract or repel specific biomolecules or cells,62 the responsive nature of the film can be

used for control release of encapsulated drugs,<sup>63</sup> for response membranes<sup>64</sup> or in sensor technologies.<sup>65</sup> For these three different states, it must, however, be considered that the swelling capacity of the PPEGMEMA/alginate multilayered film is governed by the balance between the osmotic pressure of the solution and the entropic penalty of stretching the polymer chains between the cross-linking points.

#### 4. Conclusion

In this work, three PPEGMEMA-based cationic copolymers with different average PEG side chain lengths were paired with alginate via electrostatic LbL assembly. In situ QCM-D and ellipsometry measurements were used to follow the multilayer buildup process for the three different systems, and the result shows that the thickness and structure of the multilayered film is closely correlated to the average PEG side chain length in the PPEGMEMA-based cationic copolymers. Specifically, as the average PEG side chain length increases, two main observations are noted. First, it is found that the multilayer builds up less effectively with a reduced increase in added mass during the alternating deposition of polymers. Second, the film is found to become more dissipative and exhibit a higher water content. Both of these effects are interpreted as being directly related to the average PEG side chain length, which, due to the high hydrophilicity of the PEG units, leads to a highly hydrated layer and to steric hindrance, which limits the amount of polymer adsorbed in each deposition step.

After buildup, the obtained PPEGMEMA/alginate multilayered films were cross-linked with glutaraldehyde to exhibit pH responsiveness without disintegration of the layer due to charge imbalance in the layer. It was further demonstrated how the PPEGMEMA/alginate multilayered films can be in a cationic, zwitterionic or anionic state depending on the pH, and how the transitions between these states lead to structural changes in the layer.

#### Conflicts of interest

There are no conflicts to declare.

#### Acknowledgements

We would like to acknowledge the financial support from the Independent Research Fund Denmark (grant # 6111-00102B).

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Supporting Information for

## PPEGMEMA-based Cationic Copolymers Designed for Layer-by-Layer Assembly

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#### S1 Characterization of P(PPEGMEMA-stat-AMA) Copolymers

#### S1.1 <sup>1</sup>H NMR spectra of s-PPEGMEMA and I-PPEGMEMA

The 1H NMR spectra of all three synthesized copolymers were measured to characterize the three PPEGMEMA copolymers synthesized. A Brucker 400 MHz NMR spectrometer was used, with D2O as the solvent. The TopSpin software from Brucker was used to process the NMR data obtained. The NMR spectra of s-PPEGMEMA, m-PPEGMEMA and l-PPEGMEMA are presented in Figure S1, Figure 1 and Figure S2, respectively.



Figure S1 1H NMR spectrum of s-PPEGMEMA



Figure S2 <sup>1</sup>H NMR spectrum of l-PPEGMEMA

#### S1.2 AF4 chromatograms of P(PEGMEMA-stat-AMA)

The molecular weight and polydispersity of the three P(PEGMEMA-*stat*-AMA) polymers were determined by AF4 with a Wyatt Eclipse instrument with UV (Agilent 1230 infinity, Agilent), refractive index (Optilab rex, 633 nm, Wyatt) and multi-angle light scattering (MALS) (Dawn Heleos-II, 662 nm, Wyatt) detectors. The Optilab RI detector was used to precisely determine the refractive index increment (dn/dc) values of the three polymers. A concentration series of 0.05, 0.1, 0.25, 0.5 and 1 mg/ml were used and the corresponding dRI values were plotted and fitted linearly. The slope was calculated as the dn/dc value and accurate MW were calculated accordingly.



Figure S3 AF4 chromatogram of s-PPEGMEMA



Figure S4 AF4 chromatogram of m-PPEGMEMA



Figure S5 AF4 chromatogram of l-PPEGMEMA

# S2 Estimation of Hydrated PPEGMEMA/alginate Multilayered Film Density

The volume fraction of the solvent,  $f_s$ , in the PPEGMEMA/alginate multilayered film were estimated with the BEMA model from our ellipsometry data. The hydrated PPEGMEMA/alginate multilayered film density  $\rho_f$  was estimated according to the following equation:

$$\rho_f = \rho_s f_s + \rho_p (1 - f_s)$$

Where  $\rho_s$  and  $\rho_p$  are the densities of solvent and dry polymer, respectively. Herein  $\rho_s$  is 999 g/mL, and  $\rho_p$  is 1.4 g/cm<sup>3</sup>, estimated as an average of the polymer density of PEG (1.2 g/mL) and sodium alginate (1.6 g/cm<sup>3</sup>). The hydrated PPEGMEMA/alginate multilayered film density was finally calculated to be 1080 g/cm<sup>3</sup>, 1060 g/cm<sup>3</sup> and 1040 g/cm<sup>3</sup> for s-PPEGMEMA/alginate, m-PPEGMEMA/alginate and 1-PPEGMEMA/alginate multilayered films, respectively.

#### S3 Ellipsometry

#### S3.1 Bare QCM sensor modelling

To reduce the number of layers that contribute to the optical properties of the QCM sensor substrate and simplify the modeling process, a tailor-made sensor (QSX335) with a thick opaque interlayer of titanium was used. The optical model for substrate then consists of a titanium substrate and a layer of silica. To avoid overparameterization, the thickness of silica coating was fixed as 25 nm, and only the optical constants of the titanium substrate was fitted with a B-Spline model (resolution 0.2 eV, parameterized initially from Ti optical constants from software library).



Spectroscopic Ellipsometric (SE) Data

Figure S6 QCM bare sensor Psi and Delta in air with the fitted model described (dashed line)



Figure S7 QCM bare sensor Psi and Delta in buffer with the fitted model described

#### S3.2 Ellipsometry data modelling of PPEGMEMA/alginate multilayered film

As has been discussed in the Experimental section, the PPEGMEMA/alginate multilayered film was modeled with single-component Cauchy and two-component BEMA models. Detailed Psi and Delta spectra of all three PEM films are shown in Figure S8-13. The effect of several parameters such as surface roughness, grading, and thickness nonuniformity were tested, where only the latter was found to significantly improve the fitting quality and thus included in the model.



#### Spectroscopic Ellipsometric (SE) Data



Figure S8 Ellipsometry data as well as the Cauchy modelling for s-PPEGMEMA/alginate multilayered film



#### Spectroscopic Ellipsometric (SE) Data



Figure S9 Ellipsometry data as well as the BEMA modelling for s-PPEGMEMA/alginate multilayered film
Fit Results	Optical Model
MSE = 5.941	- Layer # 2 = <u>Cauchy</u> Thickness # 2 = <u>180.73 nm</u> (fit)
Thickness $\# 2 = 180.73 \pm 0.584$ nm	A = <u>1.349</u> (fit) B = <u>0.00393</u> (fit) C = <u>0.0000</u>
$A = 1.349 \pm 0.00027572$	+ Urbach Absorption Parameters
$B = 0.00393 \pm 4.5621E-05$	Layer # 1 = <u>SiO2_JAW</u> Thickness # 1 = <u>25.00 nm</u>
% Thickness Non-uniformity = $100.00 \pm$	+ Substrate = <u>B-Spline</u>
1.117	,

# Spectroscopic Ellipsometric (SE) Data



Figure S10 Ellipsometry data as well as the Cauchy modelling for m-PPEGMEMA/alginate multilayered film







Figure S11 Ellipsometry data as well as the BEMA modelling for m-PPEGMEMA/alginate multilayered film

Fit Results	Optical Model		
MSE = 13.713 Thickness # 2 = 159.46 ± 2.948 nm A = 1.337 ± 0.00059798 B = 0.00341 ± 7.0185E-05 % Thickness Non-uniformity = 100.00 ± 8.130	- Layer # 2 = <u>Cauchy</u> Thickness # 2 = <u>159.46 nm</u> (fit) A = <u>1.337</u> (fit) B = <u>0.00341</u> (fit) C = <u>0.0000</u> + Urbach Absorption Parameters		
	Layer # 1 = <u>SiO2_JAW</u> Thickness # 1 = <u>25.00 nm</u> + Substrate = <u>B-Spline</u>		

# Spectroscopic Data At 305.787 min.



Figure S12 Ellipsometry data as well as the Cauchy modelling for I-PPEGMEMA/alginate multilayered film



## Spectroscopic Data At 305.787 min.



Figure S13 Ellipsometry data as well as the BEMA modelling for l-PPEGMEMA/alginate multilayered film

Fit Results	Optical Model			
MSE = $63.300$	<ul> <li>Layer # 2 = <u>Cauchy</u> Thickness # 2 = <u>48.87 nm</u> (fit)</li></ul>			
Thickness # 2 = $48.87 \pm 0.059$ nm	A = <u>1.500</u> B = <u>0.00500</u> C = <u>0.0000</u> <li>+ Urbach Absorption Parameters</li> <li>Layer # 1 = <u>SiO2_JAW</u> Thickness # 1 = <u>25.00 nm</u></li> <li>+ Substrate = <u>B-Spline</u></li>			

# **Experimental and Model Generated Data Fits**



Figure S14 Ellipsometry data as well as the Cauchy modelling for dry s-PPEGMEMA/alginate multilayered film in air

Fit Results	Optical Model			
MSE = $97.154$ Thickness # 2 = $29.37 \pm 0.086$ nm	<ul> <li>Layer # 2 = <u>Cauchy</u> Thickness # 2 = <u>29.37 nm</u> (fit)</li> <li>A = <u>1.500</u> B = <u>0.00500</u> C = <u>0.0000</u></li> <li>Hirbach Absorption Parameters</li> </ul>			
	Layer # 1 = <u>SiO2_JAW</u> Thickness # 1 = <u>25.00 nm</u> + Substrate = <u>B-Spline</u>			

# **Experimental and Model Generated Data Fits**



Figure S15 Ellipsometry data as well as the Cauchy modelling for dry m-PPEGMEMA/alginate multilayered film in air

Fit Results	Optical Model			
MSE = 43.426 Thickness # 2 = 17.58 ± 0.029 nm	- Layer # 2 = <u>Cauchy</u> Thickness # 2 = <u>17.58 nm</u> (fit) A = 1.500 B = 0.00500 C = 0.0000			
	+ Urbach Absorption Parameters			
	Layer # 1 = <u>SiO2_JAW</u> Thickness # 1 = <u>25.00 nm</u>			
	+ Substrate = <u>B-Spline</u>			

# **Experimental and Model Generated Data Fits**



Figure S16 Ellipsometry data as well as the Cauchy modelling for dry l-PPEGMEMA/alginate multilayered film in air

# S4 PPEGMEMA/alginate Multilayered Film Stability Before Glutaraldehyde Crosslinking

The pH stability of the m-PPEGMEMA/alginate multilayered film before crosslinking of glutaraldehyde was examined by flushing a pH 8 buffer over the prepared film. As is shown in Figure S17, an immediate disassembly of the film was observed, evidenced by a drastic decrease in frequency and dissipation shifts.



Figure S17 QCM-D Frequency and dissipation shifts after inflow of pH 8 carbonate buffer before crosslinking of glutaraldehyde

# Paper 2

# A Single-component, Cross-linked, and Surface-grafted

# Polyelectrolyte Film Fabricated by the Layer-by-layer Assembly

# Method



1. EDC/NHS cross-linking; 2. Amine quenching with m-PEG3-COOH, EDC/NHS



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# A single-component, cross-linked, and surface-grafted polyelectrolyte film fabricated by the layer-by-layer assembly method



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#### ARTICLE INFO

Keywords: Single-component polyelectrolyte film Layer-by-layer PEGMEMA QCM-D

#### ABSTRACT

In this work, we demonstrate a versatile approach for the fabrication of a single-component polyelectrolyte layer with tunable thickness and functionality. Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA) was copolymerized with either aminoethyl methacrylate (AMA) or methacrylic acid (MAA) to prepare statistical PEGMEMA copolymers carrying positive or negative charges, respectively. These polyelectrolytes were used in a conventional layer-by-layer assembly process followed by cross-linking to form a surface-grafted stable poly (PEGMEMA) layer carrying both negative and positive charges. To transform this layer into a single-component polyelectrolyte layer carrying just one type of ionizable group, the amino groups were quenched, thus leaving a single-component anionic PEGMEMA-based polyelectrolyte layer. Last, we demonstrate that compared to a zwitterionic layer, the anionic polyelectrolyte layer exhibits an enhanced protein-repelling property against bovine serum albumin (BSA).

#### 1. Introduction

pH-responsiveness

Polyelectrolyte films are thin molecular layers that consist of polymer chains with ionizable groups. The chemical composition and ionizable groups of the polyelectrolyte chains control the functional properties of the film, such as controlled permeability [1], anti-icing [2], and antifouling [3]. Polyelectrolyte films can be fabricated through different methods. For instance, desired polyelectrolytes have been chemically grafted onto a target substrate via either the "graft-onto" approach [4,5] or surface-initiated polymerization [6,7]. A more convenient and adaptable method is through physical adsorption of the polyelectrolyte chains onto the substrate, e.g., electrostatic adsorption onto charged surfaces [8,9]. However, the formed layer is typically only a few nanometers thick, showing modest surface coverage [10,11].

Layer-by-layer assembly (LbL) is an alternative method for the fabrication of relatively thick polyelectrolyte films [12–16]. Herein, a polyelectrolyte multilayered (PEM) film is prepared by the sequential adsorption of oppositely charged polyelectrolytes onto a charged surface. The desired thickness of the PEM films is then realized using the number of depositions, and it can reach up to several microns [17]. Moreover, LbL assembly is a straightforward and versatile method that allows coating surfaces of different sizes and geometries. While conventional PEM films consist of at least two different polyelectrolyte

components, only a handful of studies have reported the preparation of single-component multilayer films [18–21]. For instance, Liu et al. prepared a single-component chitosan film by fabricating a cross-linked chitosan/polyacrylic acid PEM and then removing the polyanion by overnight treatment with an alkaline solution [22,23]. Despite the simplicity of this approach, it lacks versatility, and incomplete removal of the second component is expected. As another approach, Tong et al. fabricated a poly(allylamine hydrochloride) (PAH) multilayer film in which glutaraldehyde-mediated covalent interactions promote the LbL assembly [24]. The fast Schiff base chemistry provides a feasible approach for repeated PAH deposition, driving film thickness growth. Nevertheless, this method is driven by chemical bond formation and hence suffers a low layer buildup efficiency, and a high pH value is required to minimize the electrostatic repulsion between the amino groups.

Another effective method to fabricate single-component polyelectrolyte multilayered films is based on the copolymerization of a functional monomer with a charged monomer [25]. We previously copolymerized poly(ethylene glycol) methyl ether methacrylate (PEG-MEMA) with 2-aminoethyl methacrylate (AMA). It was shown that the obtained PEGMEMA-based copolymer contains positive charges that are randomly distributed along the copolymer chain; hence, it can be paired with a given polyanion using the LbL assembly method [26]. Following

https://doi.org/10.1016/j.polymer.2020.122524

Received 4 March 2020; Received in revised form 22 April 2020; Accepted 24 April 2020 Available online 30 April 2020 0032-3861/© 2020 Elsevier Ltd. All rights reserved.

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this same approach, it is feasible to copolymerize PEGMEMA with a negatively charged monomer to prepare an anionic PEGMEMA-based copolymer. The two oppositely charged PEGMEMA-based copolymers can be paired using the LbL assembly method, yielding a polyelectrolyte film with a tunable thickness that consists of only one functional monomer.

Adopting this approach, in this work, we have fabricated a singlecomponent PPEGMEMA multilayered film. To do so, we copolymerized PEGMEMA with AMA and methacrylic acid (MAA) to prepare positively and negatively charged PEGMEMA copolymers, respectively. The two charged PEGMEMA copolymers were LbL assembled onto a preaminated silica substrate to provide a PPEGMEMA multilayered film, followed by chemical cross-linking with carbodiimide chemistry to enhance the film stability and graft the film onto the substrate. Furthermore, to obtain a truly single-component PPEGMEMA polyelectrolyte film with only negative charges, the residual amino groups in the film were quenched with a PEG-terminated carboxylic acid, m-PEG3-COOH. Finally, we examined the adsorption of bovine serum albumin (BSA) on the PPEGMEMA films.

#### 2. Experimental section

#### 2.1. Materials

PEGMEMA (number average molecular weight  $(M_n)$  of 300 g mol<sup>-1</sup>) and t-butyl methacrylate (99%) were purchased from Sigma-Aldrich Denmark and treated using neutral alumina column chromatography to remove the inhibitors before use. (2-Boc-Amino)ethyl methacrylate (t-BocAMA, 99%), diethyl meso-2,5-dibromoadipate (98%), trifluoroacetic acid (TFA, 99%), (3-aminopropyl)triethoxysilane (APTES, 99%) and copper(I) chloride (CuCl, >99%, washed sequentially with acetic acid and ethanol before use) were purchased from Sigma-Aldrich and used as received unless otherwise stated. Tris(2-dimethylaminoethyl) amine (Me6TREN, 99%) was purchased from Alfa Aesar. m-PEG3-COOH (99%) was purchased from BroadPharm and used as received. All the solvents used in this work were of HPLC grade and purchased from Sigma-Aldrich. A pH 7 phosphate buffer solution (pH adjusted to 7 with 50 mM sodium hydrogen phosphate dibasic) was used for the preparation of the polymer solutions as well as in the rinsing step between each layer deposition. Solutions containing 300 ppm (w/v) P(PEGMEMA-stat-MAA) and P(PEGMEMA-stat-AMA) were prepared for LbL assembly and filtered through 0.22 µm nylon syringe filters to remove possible aggregates and dust. Ultrapure water (Sartorius Arium® Pro ultrapure water system, resistivity of 18.2 MQ cm) was used to prepare all the solutions.

#### 2.2. Synthesis of P(PEGMEMA-stat-AMA) and P(PEGMEMA-stat-MAA)

A two-step protocol was employed to avoid the interference of the pendant amino groups to the polymerization, as formerly reported [27]. First, PEGMEMA was copolymerized with protected t-BocAMA, yielding a P(PEGMEMA-stat-tBocAMA) statistical copolymer, where all the AMA units were protected by t-Boc groups. In the second step, the t-Boc groups were removed under acidic conditions (TFA/DCM), giving the desired P(PEGMEMA-stat-AMA) copolymer. Briefly, for the synthesis of P(PEGMEMA-stat-tBocAMA), diethyl meso-2,5-dibromoadipate (7.2 mg, 0.02 mmol), PEGMEMA (M<sub>n</sub> = 300) (900 mg, 3 mmol), t-BocAMA (229.27 mg, 1 mmol) and Me6TREN (9.2 mg, 0.04 mmol) were dissolved in 5 ml of isopropanol. Dimethylformamide (0.1 ml) was added to the reaction mixture as an internal standard for later calculation of the monomer conversion and molecular weight (Mn). The reaction mixture was purged with nitrogen gas for at least 30 min, after which prewashed copper(I) chloride (1.23 mg, 0.0125 mmol) was added. The polymerization was triggered by immersing the reaction flask in a 50 °C water bath. After 16 h, the reaction was quenched by adding 5 ml of water into the solution and exposure to air. A 0.1 ml sample of the solution was

collected for proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy measurement to determine the reaction conversion. The polymer solution was then purified via dialysis (using regenerative cellulose tubing with an MWCO of 6-8 kDa, Spectrum Laboratories, Inc.) against water for 3 days. Finally, the residual water was removed via lyophilization to obtain the pure P(PEGMEMA-stat-tBocAMA) polymer. In the deprotection process, the obtained P(PEGMEMA-stat-tBocAMA) (300 mg) was dissolved in 4 ml of dichloromethane, followed by the addition of 0.5 ml of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 3 h, after which the dichloromethane was removed by evaporation. The viscous polymer residue was redissolved in 5 ml of water, and the pH was adjusted to be approximately neutral with a 1 M NaOH solution. The polymer was purified by dialysis and subsequent lyophilization, yielding a light yellow viscous liquid. The <sup>1</sup>H NMR spectra of P(PEGMEMA-stat-tBocAMA) and P(PEGMEMA-stat-AMA) are available in our previous work [26].

The P(PEGMEMA-*stat*-MAA) copolymer was synthesized in a similar manner. Instead of (2-Boc-amino)ethyl methacrylate, t-butyl methacrylate was added and copolymerized with PEGMEMA at the same stoichiometric ratio. All the reaction procedures, purification techniques, and characterization methods were the same as those used for P(PEG-MEMA-*stat*-AMA). The <sup>1</sup>H NMR spectra of P(PEGMEMA-*stat*-tBuMAA) and P(PEGMEMA-*stat*-MAA) are shown in Fig. S1. The t-butyl protection group peak (1.4 ppm) vanished after treating with TFA, confirming successful deprotection. For nomenclature convenience, hereafter, we name P(PEGMEMA-*stat*-MAA) and P(PEGMEMA-*stat*-AMA) as PPEG-MEMA(-) and PPEGMEMA(+), respectively.

The <sup>1</sup>H NMR (Bruker 400 MHz) spectra were used to calculate the conversion and number average molecular weight (M<sub>n</sub>). To do so, 0.1 ml samples of the reaction mixture were collected before and after the polymerization process. The <sup>1</sup>H NMR spectra of both samples were taken using deuterated dimethyl sulfoxide (DMSO) as the solvent. The integral ratios of  $\delta$  6.0 ppm (monomer double bond (vinyl)) to  $\delta$  7.9 ppm (DMF) before (r<sub>1</sub>) and after (r<sub>2</sub>) polymerization were calculated to compute the monomer conversion (c) via the simple relation  $c = 1 - r_2/r_1$ . M<sub>n</sub> was then calculated using:

$$M_{theo} = M_i + M_{mon}$$

where  $M_{\rm i}$  and  $M_{\rm mon}$  are the molecular weights of the initiator and the monomer, respectively.

To independently determine the number average molecular weight  $(M_n)$ , weight average molecular weight  $(M_w)$ , and polydispersity index (PDI) of the copolymers, asymmetric flow field-flow fractionation (AF4) was performed using a Wyatt Eclipse instrument with UV (Agilent 1230 infinity, Agilent), refractive index (Optilab rex, 633 nm, Wyatt) and multiangle light scattering (MALS) (Dawn Heleos-II, 662 nm, Wyatt) detectors. A frit-inlet channel equipped with a regenerated cellulose membrane (MWCO 5 kDa, Millipore) and a 350  $\mu m$  width spacer were used as the separation channel. Each sample was analyzed with a constant detector flow of 0.5 mL min $^{-1}$ , and a cross-flow that decreased exponentially from 3 mL min<sup>-1</sup> to 0 in 20 min. A 50 mM PBS pH 7.4 buffer solution was prepared and filtered with a 0.1 µm membrane (Millipore) for use as the eluent. The samples were dissolved in the PBS buffer to a concentration of 5 mg/ml, and the injection volume was set as 100 µL. A refractive index increment (dn/dc) value of 0.12 was determined and used in the calculation of the molecular weight and polydispersity, as described in our previous work [26]. The Astra software (Wyatt, version 7.1.3.15) was used for data analysis and calculation of the molecular weight with the Debye plot using the Zimm formula [28]. The chromatogram of PPEGMEMA(+) is shown elsewhere [26], while that of PPEGMEMA(-) is provided in the Supporting Information (Fig. S2).

#### 2.3. Quartz crystal microbalance with dissipation monitoring (QCM-D)

#### 2.3.1. Instrumentation and theory

The LbL assembly and pH-responsiveness of the PPEGMEMA (+)/PPEGMEMA(-) multilayer films were monitored with QCM-D (Q-Sense E1, Biolin Scientific, Gothenburg, Sweden) using silica-coated sensors (QSX 303, Biolin Scientific). In a typical QCM-D experiment, a quartz crystal sensor undergoes an oscillating voltage, leading to the corresponding oscillation at its resonance frequency (F). In addition, the sensor oscillation amplitude is monitored throughout the decay, and the dissipation factor (D) is calculated as the dissipated energy divided by the total stored energy. For thin, uniform and rigid films, the frequency shifts are proportional to the adsorbed mass per unit area, which is modeled by the Sauerbrey equation. However, the Sauerbrey equation provides a valid estimation only if the ratio of dissipation and normalized frequency shifts is sufficiently small (i.e., if  $(\Delta D_n)/(-\Delta f_n/n)$  is smaller than  $4 \times 10^{-7}$  Hz<sup>-1</sup>) so that the film can simplistically regarded as rigid [29]. In contrast, for soft and highly hydrated polymeric films, the so-called effective coupled mass depends on how the oscillatory acoustic wave propagates through the attached film. Consequently, the coupled water (either bound or unbound) and the viscous drag force will additionally contribute to the frequency shifts. Under this condition, the viscoelastic Voigt model provides a better estimation of the adsorbed mass, where the adhered film is represented by a layer of uniform thickness and density with distinct viscous and elastic components. Accordingly, the frequency and dissipation shifts are related to the properties of the film and the medium following [30]:

$$egin{aligned} & \Delta f \ f \ = \ - \ \displaystyle rac{d_f 
ho_f}{d_q 
ho_q} \left( 1 - \eta_0 
ho_0 imes rac{(\eta_f / 
ho_f) \omega^2}{\left( \mu_f^2 + \omega^2 \eta_f^2 
ight)} 
ight. \ & \Delta D \ = \ \displaystyle rac{d_f}{d_q 
ho_q} \left( \eta_0 
ho_0 imes rac{\mu_f \omega}{\mu_f^2 + \omega^2 \eta_f^2} 
ight) \end{aligned}$$

where  $\omega$  is the angular frequency of the oscillation,  $\eta_0$  and  $\rho_0$  are the viscosity and density of the medium, respectively, and  $d_q$  and  $\rho_q$  are the thickness and density of the quartz crystal resonator, respectively. To avoid overparameterization, the medium density, medium viscosity, and film density are estimated and then treated as fixed parameters in the model. Therefore, the thickness (d<sub>f</sub>), viscosity ( $\eta_f$ ) and shear modulus ( $\mu_f$ ) of the polymeric film are obtained by fitting the Voigt model to the measured shifts in frequency and dissipation for different overtones (3rd, 5th and 7th). The instrument software (QSense® Dfind, v1.1, Biolin Scientific) was employed for modeling the data. The density of the film ( $\rho_f$ ) was estimated to be 1040 kg m<sup>-3</sup>. The density and viscosity of water at 23 °C (provided in the software library) were used for the medium.

#### 2.4. LbL assembly

The sensor underwent a pre-amination process with APTES before the LbL assembly [31]. To do so, the sensor was rinsed with copious amounts of ethanol and water, dried, and then plasma-cleaned (PDC-32G plasma cleaner, Harrick Plasma) in water vapor with a constant pressure of 0.5 Torr for 1 min. The clean sensor was placed in an evacuated desiccator with a 50% (v/v) APTES/toluene solution for 18 h to allow for the deposition of APTES onto the surface. Thereafter, the sensor was rinsed with copious amounts of toluene and ethanol and dried with compressed air. The sensor was then immediately mounted in the QCM-D module, and the measurement was started at 23 °C using a 75  $\mu$ L/min flow of phosphate buffer (pH 7). Upon achieving a stable baseline for all the harmonics, PPEGMEMA(+) and PPEGMEMA(-) solutions were alternately loaded (30 min for each layer) and rinsed (20 min for each layer) with buffer to obtain 7 bilayers in total. To cross-link the fabricated film, a 10 mg/mL EDC/NHS solution was flowed over the sensor for 12 h, after which the chamber was rinsed with pH 7 buffer to remove the residual EDC/NHS. To further quench the amino groups and remove the positive charges on the film, an m-PEG3-COOH solution (10 mg/ml m-PEG3-COOH, 10 mg/ml EDC/NHS) was flowed over the sensor for 12 h. To test the pH stability, NaCl solutions at pH 2.5, 7 and 10 (100 mM, pH-adjusted with HCl/NaOH) were loaded to perform 3 consecutive pH cycles.

#### 2.5. Test of BSA repellence

To test the antifouling property of the film, the adsorption of BSA on the films was investigated. First, a baseline was obtained using a pH 7.4 phosphate buffer, after which a 5% w/w BSA solution (50 mg/mL) was flowed over the sensor for 20 min. Subsequently, the chamber was rinsed with pH 7.4 phosphate buffer to remove unbound BSA. The adsorbed mass of BSA was estimated via the Sauerbrey relation [32],  $\Delta m = -\frac{C\Delta f}{n}$ , where  $\Delta m$  is the absorbed BSA mass,  $\Delta f$  is the frequency shift, n is the overtone number (1, 3, 5, ...,13), and C (17.7 ng/cm<sup>2</sup>) is the mass-sensitivity constant for the 5 M Hz quartz crystal. The frequency shift of the third overtone was used for the mass calculation.

#### 3. Results and discussion

#### 3.1. Synthesis of PPEGMEMA(+) and PPEGMEMA(-)

PEGMEMA-based polycation (PPEGMEMA(+)) and polyanion (PPEGMEMA(-)) were synthesized with a statistical copolymerization process by ATRP, in which primary amino groups and carboxyl groups are distributed randomly along the copolymer chains, respectively. The chemical structures of the synthesized copolymers are presented in Fig. 1 and a summary of their characteristics are given in Table 1. The amount of acidic or basic groups on each of the two polymers is 25%. The molecular weights of the two copolymers were measured with AF4, and the  $M_n$  values of the two polymers exhibit an overall good agreement with those obtained from <sup>1</sup>H NMR spectroscopy. The PDI values of the two polymers are approximately 1.7, which is relatively high for ATRP polymerization. However, this high value is not a concern with respect to the LbL assembly process.

#### 3.2. LbL assembly of PPEGMEMA(+)/PPEGMEMA(-)

The LbL assembly of PPEGMEMA(+) and PPEGMEMA(-) was monitored in situ with QCM-D. The shifts in the resonance frequency (F) and dissipation factor (D) resulting from multilayer deposition are presented in Fig. 2. The resonance frequency relates to the total (effective) mass coupled with the sensor, including the deposited polymer chains and the associated water content. The dissipation factor is, on the other hand, a semiquantitative measure of the layer conformation; i.e., a higher dissipation factor corresponds to a more viscoelastic layer. The odd and even layer numbers refer to the deposition of the PPEGMEMA(-) and PPEGMEMA(+) layers, respectively. Overall, 7 bilayers of the PEG-MEMA copolymers were deposited on the silica sensor. Following the deposition steps, general trends of an increase in the dissipation factor and decrease in the resonance frequency are observed, together indicating consistent polymer mass deposition on the surface. The largest frequency and dissipation shifts are observed for the first deposited bilayer, which can be attributed to the high positive charge density of the APTES-functionalized silica surface. However, after the first couple of bilayer depositions, the QCM-D shifts show a rather linear dependence on the number of layers indicating a stable growth of the PPEG-MEMA multilayered film. The overall frequency shift for the 7 bilayers is approximately -70 Hz, and the dissipation shift value is approximately 9.5, yielding a  $\Delta D/(-\Delta f)$  value of approximately  $0.14 \times 10^{-6}$ . This ratio indicates a rather viscoelastic behavior that can be attributed to the hydrated nature of the film due to the abundant presence of the PEG



Fig. 1. (a) Structure of cationic PPEGMEMA(+); (b) structure of anionic PPEGMEMA(-); and (c) structure of the mPEG3-COOH used to quench the amino groups in the multilayered films.

#### Table 1

Properties of the synthesized charge-bearing PPEGMEMA copolymers.

	Polymer Compo	sition						
	PEGMEMA	AMA	MAA	Mn <sup>a</sup> (kDa)	Mn <sup>b</sup> (kDa)	PDI <sup>b</sup>	Amine content (DP%)	Methacrylic acid content (DP%)
PPEGMEMA(+)	135	45	-	46.7	38.3	1.71	25%	_
PPEGMEMA(-)	99	-	33	32.5	29.0	1.74	-	25%

<sup>a</sup> Determined with <sup>1</sup>H NMR spectroscopy.

<sup>b</sup> Determined with AF4.

units [33,34]. This observation is further in accordance with our previous study [26], where relatively large dissipation shifts were observed.

The viscoelastic Voigt model was fitted to the frequency and dissipation shifts to estimate the acoustic thickness of the prepared PEM film. The overall thickness of the 7-bilayer PEM film is approximately 17 nm at the conditions where the layer is fabricated (phosphate buffer at pH 7). In a similar manner, the acoustic thickness demonstrates a rather linear dependence on the layer number after the first deposited bilayer. The first deposited bilayer shows an estimated thickness of approximately 6 nm, followed by a steady thickness growth of approximately 1.5 nm per bilayer.

#### 3.3. Cross-linking the PPEGMEMA(+)/PPEGMEMA(-) films

Weak polyelectrolyte multilayer films are subject to disintegration (dissolution) in response to pH variations due to the consequent charge imbalance in the film [35–37]. Fig. 3a shows the QCM-D data corresponding to the pH stability test on the prepared PPEGMEMA (+)/PPEGMEMA(-) multilayered film. Accordingly, when the pH is switched from 7 to 2.5, a drastic increase in the frequency and decrease in the dissipation are found. In addition, the frequency and dissipation shifts are completely irreversible, since the initial values of frequency and dissipation are not restored when the pH is returned to 7. Repeated pH cycling confirms the irreversible mass loss and disintegration of the PPEGMEMA(-)/PPEGMEMA(-) film, indicating the instability of the PEM film due to the positive charge imbalance produced when the pH is changed from 7 to 2.5.

To enhance the stability of the PEM film, the multilayered film can be chemically cross-linked using EDC/NHS amidation chemistry. EDC/NHS is a highly efficient cross-linker that catalyzes the amide formation reaction between the carboxyl and amino groups in the film. In addition, amidation between the APTES-treated substrate and the first PPEG-MEMA(-) layer is expected to graft the entire film onto the substrate. Hence, a surface-grafted and cross-linked PEM with enhanced stability should be obtained. The film stability towards pH variations was examined again after the film was exposed to a 10 mg/ml EDC/NHS solution for 12 h prior to any change in pH after the LbL assembly process (Fig. 3b). When the pH is decreased from 7 to 2.5, an increase in the dissipation and decrease in the frequency were observed, indicating a swelling behavior of the film. This result is in accordance with previous reports regarding the pH-responsive behavior of cross-linked weak polyelectrolyte multilayers [23,26,35]. When the pH is returned from 2.5 to 7, a decrease in the dissipation and an increase in the frequency are found, which together indicate the shrinkage of the film back to its initial state. This pH cycling was repeated three times, and despite a minor hysteresis, a reversible and repeatable swelling-shrinking process is observed, indicating a significantly more stable and surface-grafted PEM film, as well as a pH-responsiveness of the cross-linked multilayered film.

To test the stability of the film under alkaline conditions, the pH was repeatedly cycled between 7 and 10. Accordingly, increasing the pH from 7 to 10 results in an increase in the dissipation and a decrease in the frequency, indicating a swelling behavior of the film. Decreasing the pH back to 7 gives rise to a decrease in the frequency and an increase in the dissipation, suggesting shrinkage of the multilayered film. It is noteworthy that the QCM-D shifts when the pH is changed from 10 to 7 are larger than those when the pH is increased from 7 to 10, which might be due to a loss of unbound PPEGMEMA polymer chains and/or a structural reorganization of the film. Nevertheless, after the first pH cycle, the film exhibits only minor hysteresis and repeatable swelling-shrinking behavior when the pH is shifted between 7 and 10, implying a high film stability under alkaline conditions.

The observed dual pH-responsiveness of the film indicates the presence of unreacted amine and carboxyl groups in the film. At pH 7, where the LbL assembly is conducted, the film is found in a charge-neutral state where the number of charged amino groups and carboxyl groups within the film are comparable. At this "zwitterionic" state, the film adopts a collapsed conformation. By decreasing the pH from 7 to 2.5, the amine and carboxyl groups undergo a protonation process, leading to a net positive charge imbalance in the film. The film thus exhibits an overall cationic state, and hence, a swollen conformation is found due to the osmotic pressure difference, as discussed in our previous work [26]. When the pH is increased from 7 to 10, the deprotonation of amine and carboxyl groups leads to a net negative charge imbalance within the film. Consequently, the film exhibits an overall anionic state and adopts a swollen conformation.



**Fig. 2.** QCM-D monitoring of LbL of PPEGMEMA(+)/PPEGMEMA(-) in pH 7 phosphate buffer; (a) frequency and dissipation shifts; (b) film thicknesses obtained by Voigt modeling.

#### 3.4. Transformation from PPEGMEMA(+)/PPEGMEMA(-) to a singlecomponent PPEGMEMA(-) polyelectrolyte film

As described in Fig. 3, the stabilized (cross-linked) PPEGMEMA (+)/PPEGMEMA(-) multilayer film contains both amine and carboxyl groups, and it will thus demonstrate a zwitterionic state under neutral pH conditions. To obtain a genuine single-component polyelectrolyte film similar to a layer consisting of just one type of copolymer (PPEG-MEMA(-)), the excess amino groups in the film are quenched via the same EDC/NHS chemistry utilized in the cross-linking process. To do so, a PEGylated carboxylic acid (m-PEG3-COOH) was dissolved into the EDC/NHS solution and was loaded into the QCM chamber for 12 h. Consequently, the amino groups were replaced by short PEG units via amide formation.

Fig. 4 displays the QCM-D data of the pH cycles performed on the film after the amine quenching process. By decreasing the pH from 7 to 2.5, an increase in the frequency and a decrease in the dissipation were observed, probably due to the removal of the unbound cross-linking agent and a reorganization of the film. Nevertheless, further cycling of the pH between 7 and 2.5 results in negligible shifts of the frequency (~1 Hz) and dissipation (<0.1). The loss of pH-responsiveness and the swelling of the film under acidic conditions confirm the successful



**Fig. 3.** Frequency (blue circle) and dissipation (red square) shifts of PPEG-MEMA(+)/PPEGMEMA(-) film during repeated pH cycles from 2.5 to 7 for (a) a non-cross-linked system and (b) a system cross-linked with EDC/NHS for 12 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

quenching of the amino groups within the film. In contrast, the film shows pH-responsiveness and swelling under alkaline conditions, a behavior similar to that observed before the amine quenching process. Therefore, by means of amine quenching, the zwitterionic film is converted into a single-component, negatively charged PPEGMEMA polyelectrolyte film.

#### 3.5. Investigation of BSA repellence

To demonstrate the functional differences between the singlecomponent PEM and the two-component PEM (carrying both negative and positive charges), we finally studied the adsorption of BSA to the single-component and two-component films. PEG and its derivatives have been extensively investigated as promising materials for proteinresistant coatings [38–40]. Since proteins contain charged groups, the electrostatic state of a coating can contribute to the protein-coating interactions [41]. At a neutral pH, BSA carries a net negative charge, and by choosing BSA we therefore can investigate the potentially higher protein repellence of the anionic single-component PEM compared to the zwitterionic two-component PEM. To do so, a 5% (w/w %) BSA



**Fig. 4.** Frequency (blue circle) and dissipation (red square) shifts of PPEG-MEMA(-) polyelectrolyte film as a function of pH during pH cycles from 2.5 to 7 after amine quenching with m-PEG3-COOH. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

solution in pH 7.4 phosphate buffer was loaded into the QCM-D cell for 20 min, followed by 20 min of rinsing with the phosphate buffer. Fig. 5 illustrates the frequency shifts resulting from the adsorption of BSA on bare silica, the two-component zwitterionic film (before amine quenching), and the negatively charged single-component film (after amine quenching). As a reference substrate, the adsorption of BSA is first assessed on a bare silica sensor, which indicates a frequency decrease of approximately 32 Hz, corresponding to an adsorbed mass of approximately 570 ng/cm<sup>2</sup>. For the two-component PPEGMEMA film (before amine quenching), a frequency decrease of approximately 11 Hz is found, which corresponds to an adsorbed mass of approximately 190 ng/cm<sup>2</sup>. Finally, for the negatively charged single-component PPEG-MEMA film (after amine quenching), a frequency shift of approximately -5 Hz is observed, corresponding to an adsorbed BSA mass of 90  $ng/cm^2$ . Accordingly, the multilayered films, in general, reduce BSA adsorption compared to that on bare silica. However, it is also shown that the positive charges in the zwitterionic PEM have a negative impact on the BSA repellence, and the removal of the amino group leads to a further reduction in BSA adsorption. To this end it should be noted that the positively charged amino group were neutralized by the incorporation of short PEG units from m-PEG3-COOH, which might add to the BSA repellant properties of the single-component PPEGMEMA film.

#### 4. Conclusion

In this work, we proposed a versatile approach to prepare a surfacegrafted single-component polyelectrolyte film with a tunable film thickness. Two oppositely charged PEGMEMA copolymers were first synthesized and then LbL assembled. In situ monitoring of the LbL assembly by QCM-D demonstrated successful layer buildup, with an approximately 17 nm film obtained in 7 bilayers. The multilayered film was then cross-linked using EDC/NHS chemistry to chemically graft onto the aminated substrate and enhance the film stability towards pH variations. The cross-linked film exhibited swelling under both acidic and basic conditions without experiencing disintegration of the film or significant mass loss. To obtain a truly single-component PPEGMEMA polyelectrolyte film, the amino groups in the film were quenched with m-PEG3-COOH, leaving behind only the negatively charged carboxyl groups. After the quenching process, the film shows no swelling under acidic conditions, while its pH-responsiveness under alkaline conditions is retained, indicating successful elimination of the amino groups. The



**Fig. 5.** Surface fouling test with 5% (w/w %) BSA adsorption: (a) frequency shifts obtained from QCM-D and (b) BSA mass absorbed calculated accordingly for bare silica (gray), the cross-linked PPEGMEMA coating before amine quenching (blue) and after amine quenching with m-PEG3-COOH (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

single-component PPEGMEMA polyelectrolyte film also demonstrated a relatively high resistance against BSA adsorption. In conclusion, the proposed method might serve as a convenient and efficient approach to fabricate a functional single-component polyelectrolyte film with tunable thickness by using the LbL assembly approach.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### CRediT authorship contribution statement

**Tao Jiang:** Data curation, Visualization, Writing - original draft. **Saeed Zajforoushan Moghaddam:** Writing - review & editing, Supervision. **Esben Thormann:** Conceptualization, Methodology, Writing review & editing, Supervision.

#### Acknowledgement

We would like to acknowledge the financial support from the Independent Research Fund Denmark (grant # 6111-00102 B).

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.polymer.2020.122524.

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Supporting Information for

# A Single-component, Cross-linked, and Surface Grafted Polyelectrolyte Film Fabricated by the Layer-by-layer Assembly Method

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# **S1** Characterization of P(PEGMEMA-*stat*-MAA)

# **S1.1** <sup>1</sup>H NMR Spectroscopy



Figure S1<sup>1</sup>H NMR of P(PEGMEMA-stat-MAA), with t-butyl protection group (bottom) and after deprotection (top)

<sup>1</sup>H NMR Spectroscopy was utilized to confirm the structure of the synthesized P(PEGMEMA-*stat*-MAA). The spectra were measured with a Brucker 400 MHz NMR spectrometer. D2O was used as the solvent. The TopSpin software from Brucker was used to process the NMR data obtained. It can be clearly observed that the t-butyl group peak (1.4ppm, bottom) was removed after deprotection (top).



Figure S2 AF4 chromatogram of P(PEGMEMA-stat-MAA)

A Wyatt Eclipse instrument with UV (Agilent 1230 infinity, Agilent), refractive index (Optilab rex, 633 nm, Wyatt) and multi-angle light scattering (MALS) (Dawn Heleos-II, 662 nm, Wyatt) detectors was utilized for determination of molecular weight and polydispersity of P(PEGMEMA-*stat*-MAA). A refractive index increment (dn/dc) value of 0.12 was used, as was determined previously,<sup>1</sup> and accurate MW were calculated accordingly with an Astra 7.1 software.

# References

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# Paper 3

# A cross-linked and pH-responsive Polyelectrolyte Multilayer Film with Tuneable Interfacial Properties

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## Abstract

We report a simple and versatile approach to fabricate a cross-linked polyelectrolyte multilayer film with a chemically modifiable aminated outer surface. A polyelectrolyte multilayer (PEM) film comprising poly (2-aminoethyl methacrylate) (PAMA) and polymethacrylic acid (PMAA) was first prepared by the layer-by-layer assembly method, with a partially tert-butyloxycarbonyl (Boc) protected random copolymer, PAMA-co-PBocAMA, as the final layer. The obtained PEM film was cross-linked with EDC/NHS reagents and was found responsive to both acidic (pH 2.5) and basic (pH 9) pH conditions. The free amino groups in the PEM film was afterward quenched via an amidation process with acetic acid in the presence of EDC/NHS as the cross-linker. After cross-linking, the responsive swelling of the film towards pH 2.5 was found to be significantly attenuated, while the pH swelling behavior towards pH 9 remained unperturbed. To eventually obtain the PAMA/PMAA PEM film with free amino groups selectively situated on the outer layer, the film underwent a deprotection process to unprotect the amino groups in the outer layer. These amino groups were later used to modify the outer layer with short alkyl and poly (ethylene oxide) chains to tune the surface energy of the polyelectrolyte multilayer film. Different contact angles were observed as an indication of successful modification, while a similar pH-responsiveness behavior as before surface modification indicates little perturbation of the internal structure of the film.

**Keywords**: Polyelectrolyte multilayer film; Film interfacial modification; EDC/NHS crosslinking; pH-responsive thin film

### 1. Introduction

Polymer thin films for surface modification are essential in a wide range of applications including surface antifouling,[1] friction tuning,[2] anti-icing,[3] and controlled cell growth.[4] A broad spectrum of methods is available for the fabrication of polymer-coated surfaces,[5–8] among which the layer-by-layer (LbL) assembly is regarded as a facile, flexible, and versatile method.[8–10] In a typical LbL process, two oppositely charged polyelectrolytes are alternately deposited onto the target surface, giving rise to a polyelectrolyte multilayer (PEM) film. A PEM film has distinguished physicochemical properties and functionality within the film (bulk properties) and at the film surface (interfacial properties). Examples of bulk properties include film viscoelasticity,[11,12] hydration level,[13] conductivity,[14] and permeability.[15] In contrast, the outer surface of the film serves as a medium where direct interaction of the film with the ambience occurs. Therefore, the interfacial properties are associated with the performance of the films serving as hydrophilicity tuning[16,17] and selective adsorption.[18]

It is of interest, from both fundamental and applied points of view, to develop PEM films where the interfacial properties can be tuned systematically. In the literature, several approaches have been developed to selectively tune the interfacial properties of PEM films. In one approach, the LBL process is terminated with a functional layer that provides a desired interfacial functionality.[19–22] As an example, materials with tailored hydrophilicity have been employed on top of a PEM film to prepare either superhydrophilic or superhydrophobic outer-surface.[17,23,24] For instance, Zhao et al. coated silver aggregates on the matrix of a PEM film by electrodeposition and prepared a superhydrophobic surface.[25]

A more versatile approach to modify the PEM outer surface is via in-situ post-assembly modification by chemical interactions. In this approach, the outer layer of the PEM film is chemically modified with reactive functional groups, and functional top-layer materials are introduced utilizing the corresponding chemistry. For example, Delgado et al prepared a PEM film comprising thiol groups in the outer layer and utilized the chemical reactivity of thiol groups to bind various functional materials on top of the coating.[18] This approach shows higher flexibility and is compatible with various materials of desired functionalities. Furthermore, the outer layer modification process, if under proper control, does not interfere with the bulk property of the PEM film.

Adopting this approach, we hereby demonstrate a simple and versatile method to prepare a PEM film with a chemically tunable outer layer. To do so, a PEM film comprising poly (2-amino)ethyl methacrylate (PAMA) and polymethacrylic acid (PMAA) was first prepared by LbL assembly. Afterward, a partially protected PAMA-co-PBocAMA random copolymer was deposited as the outer layer of the film. The film was then stabilized by a chemical cross-linking catalyzed by EDC/NHS, followed by quenching of the remaining amino groups in the film. With this step, the number of remaining amino groups is minimized in the bulk film, so that later surface modification draws little effect on the chemical composition in the bulk film. Finally, the Boc groups in the outer layer were removed in trifluoroacetic acid, giving rise to a cross-linked PEM film with reactive amino groups situated selectively in the outer layer. The chemical composition of the obtained PEM film can be modified by an amidation reaction using carboxylic acids comprising desired functionalities. As an illustration of the principle, two carboxylic acids with comparable chain length yet different hydrophilicity, namely m-PEG3-COOH and undecanoic acid, were coupled to the surface, and different contact angles of the modified films were observed as evidence of the successful outer layer modification.

### 2. Experimental

### 2.1 Materials

(2-Boc-amino)ethyl methacrylate (BocAMA, 99%), tert-Butyl methacrylate (tBuMA, 98%, passed through neutral alumina column to remove inhibitor immediately before use), ethyl  $\alpha$ bromoisobutyrate (EBiB, 98%), trifluoroacetic acid (TFA, 99%), (3-aminopropyl)triethoxysilane (APTES, 99%), N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA, 99%), copper(I) chloride (CuCl, >99%, washed sequentially with acetic acid and ethanol before use), N-(3dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 99%), N,N'-Dicyclohexylcarbodiimide (DCC, 99%), N-hydroxysuccinimide (NHS, >98%), and undecanoic acid (99%) were all purchased from Sigma Aldrich and used as received unless otherwise stated. m-PEG3-COOH (98%) was purchased from BroadPharm, USA and used as received. All the solvents used in this work were of HPLC grade from Sigma Aldrich. Ultra-pure water (Sartorius Arium<sup>®</sup> pro ultrapure water system, resistivity of 18.2 M $\Omega$  cm) was used to prepare all the solutions. Citric acid (>99.5%), sodium phosphate monobasic dihydrate (99%) and sodium phosphate dibasic dihydrate (99%) were purchased from Sigma Aldrich and used as received for buffer preparation. To prepare pH 4.4 citric acid/PBS buffer (used for LbL assembly), 1.074 g of citric acid and 1.57 g of Na<sub>2</sub>HPO<sub>4</sub>•2H<sub>2</sub>O were dissolved in water to prepare a 100 ml solution. PBS buffers used in cross-linking and pH cycles were prepared by adjusting the pH of the 50 mM NaH<sub>2</sub>PO<sub>4</sub> solution with either 1M HCl or NaOH. All the buffers were vacuum-filtered with Millipore 0.1 µm regenerated cellulose membrane and degassed under vacuum immediately before use. All the polymer solutions used were filtered with a 0.22 µm nylon syringe filter and degassed under vacuum before use.

#### 2.2 Synthesis of PAMA, PMAA, and PAMA-co-PBocAMA

Poly(2-aminoethyl methacrylate) (PAMA) and poly(methacrylic acid) (PMAA) were synthesized by atom transfer radical polymerization (ATRP). To eliminate the deactivation of the Cu catalyst by the carboxyl and amino groups, a two-step protection-deprotection scheme was adopted.[26,27] The protected PBocAMA and PtBMAA were first synthesized and further deprotected under acidic conditions (TFA/DCM) to yield the desired PAMA and PMAA polymers. In addition, the PBocAMA polymer underwent a short-time deprotection process (10 min) to yield the partially protected PAMA-*co*-PtBocAMA copolymer. Scheme 1 shows the structures of the polyelectrolytes used in this work. The three polyelectrolytes obtained were characterized by <sup>1</sup>H NMR and AF4. The molecular weights and the polydispersity indices (PDI) are presented in Table 1. Detailed polymerization procedures and characterizations are provided in the supporting information (Section S1).

	Mn¹ (kDa)	Mn² (kDa)	PDI <sup>2</sup>	BocAMA content (DP%)		
PAMA	11.9	11.1	1.26	-		
ΡΜΑΑ	6.1	6.5	1.49	-		
PAMA- <i>co</i> -PBocAMA	17.7	16.5	1.26	63%		

Table 1 Properties of the synthesized polymers

1. Determined with <sup>1</sup>H NMR; 2. Determined with AF4

## 2.3 Preparation of PMAA/PAMA PEM film with chemically modifiable amine outer layer

The PMAA/PAMA PEM film with an aminated outer layer was prepared in the following steps.

First, the PEM film was obtained by LbL assembly monitored with simultaneous QCM-D and spectroscopic ellipsometry utilizing a silica-coated sensor (QSX 335, Biolin Scientific). The sensor was first aminated with APTES before use.[28] For that, the sensor was rinsed with a copious amount of ethanol and water, dried, and then plasma-treated (PDC-32G plasma cleaner, Harrick

Plasma) in water vapor with a constant pressure of 0.5 Torr for 1 min. Afterward, the sensor was placed in a vacuumed desiccator with a 50% (v/v) APTES/toluene solution for 18 h. After rinsed with copious amounts of toluene and ethanol and dried with compressed air, the sensor was immediately mounted into the QCM-D module (QSense ellipsometry module, QELM 401, Biolin Scientific, Gothenburg, Sweden) and the measurement was started at 23 °C under a 75  $\mu$ L/min flow rate of citric acid/phosphate buffer at pH 4.4. After obtaining a stable baseline for all the harmonics, PMAA and PAMA solutions (100 ppm) were alternately loaded (15min) and rinsed (10min) with buffer. Finally, PAMA-*co*-PBocAMA was deposited as the last layer (14th layer).

After LbL assembly, the film was cross-linked by an inflow of 5 mg/ml EDC/NHS solution for 2h. To test the pH stability, pH 2.5, 4.5 and 9 solutions (50 mM NaH<sub>2</sub>PO<sub>4</sub> solution, pH adjusted to the designated values with 1M NaOH and HCl) were loaded. In all, three consecutive pH cycles were conducted. Afterward, the amino groups in the PEM film were quenched following a similar protocol we have utilized in our previous work.[29] To do so, a solution of 5 mg/ml EDC/NHS, 5  $\mu$ l/ml acetic acid was flowed into the cell repeatedly for a total of 4 times (2h × 2, plus 12h × 2). The first two quenching processes were conducted with a time duration of 2h each, while the last two quenching processes were elongated to 12h each.

Finally, to remove the Boc protection groups on the outer layer, the sensor was immersed in a solution containing 0.5 ml TFA and 4 ml DCM. Rinsing with copious ethanol and drying under compressed air were performed to remove residual chemicals on the sensor.

### 2.4 Surface modification with undecanoic acid and m-PEG3-COOH

The outer layer of the film is modified with either the hydrophobic undecanoic acid or the hydrophilic m-PEG3-COOH. To do so, undecanoic acid (186 mg, 1 mmol) or m-PEG3-COOH

(192 mg, 1 mmol) was first dissolved in 2 ml dichloromethane. The solution was then added dropwise under stirring, into a solution of DCC (309 mg, 1.5 mmol) in dichloromethane (3 ml). The reaction mixture was allowed under stirring for 5 min after the acid solution was completely added. The sensor with the prepared film was immersed in the solution for 12h. After the modification, the sensor was washed thoroughly with ethanol and dried with compressed airflow.

### 2.5 Techniques

## 2.5.1 In-situ QCM-D and Spectroscopic Ellipsometry

The LbL assembly and the pH-responsiveness of PMAA/PAMA(PAMA-*co*-PBocAMA) multilayered films were monitored simultaneously with QCM-D and ellipsometry. The QSense ellipsometry module (QELM 401, Biolin Scientific, Gothenburg, Sweden) designed with two optical windows incorporated was used, allowing for *in-situ* ellipsometry measurements on the QCM-D sensor. A silica-coated sensor with an optically opaque titanium substrate layer (QSX 335, Biolin Scientific) was used. For the QCM-D experiment, the shift in the resonance frequency (F) and dissipation factor (D) were recorded. The instrument software (Dfind, Biolin Scientific) was employed for data analysis. Both Sauerbrey[30] and Voigt[31] models were employed for estimation of the film thickness as a comparison. The Sauerbrey model follows a simple linear correlation of the adsorbed mass to the frequency, and is applicable to thin and rigid films; in contrast, the Voigt model provides a more accurate estimation of the adsorbed mass of a hydrated and viscoelastic film. A film density of 1200 kg·m<sup>-3</sup> was used, considering the highly impact and rigid nature of the film. The density and viscosity of water at 23 °C (from the software library) was used for the medium.

A spectroscopic ellipsometer (M2000, JA Woollam Co., USA) was used as a combination with QCM-D. Accordingly,  $\Psi$  (amplitude ratio) and  $\Delta$  (phase shift) spectra were collected (wavelength

range: 250 - 1000 nm, angle of incidence of  $70^{\circ}$ ). The instrument software (CompleteEASE, JA Woollam Co., USA) was employed for modeling. The bare sensor was first modeled as a pseudosubstrate consisting of a silica coating (25 nm, tabulated optical constants) and a thick, optically opaque titanium substrate. The optical constants of the Ti substrate were fitted with a B-Spline model (resolution 0.2 eV, parameterized initially from tabulated Ti optical constants). The fitted parameters were fixed in the modeling of the adsorbed film. The deposited film was regarded as a transparent and homogeneous layer with no adsorption (k=0); then, the film thickness and the refractive index (n) was fitted with the Cauchy model[32]:

$$n(\lambda) = A + \frac{B}{\lambda^2}$$

To estimate the water content, the film was modeled as a two-component layer consisting of a dry polymer (A = 1.5 and B = 0.005)[33,34] and water with tabulated optical constant. The volume fraction of water ( $f_w$ ) was then estimated according to the Bruggeman Effective Medium Approximation (BEMA)[35]:

$$0 = f_w \frac{n_w^2 - n^2}{n_w^2 + 2n^2} + (1 - f_w) \frac{n_p^2 - n^2}{n_p^2 + 2n^2}$$

where *n* is the refractive index of the hydrated film,  $f_w$  is the volume fraction of water, and  $n_w$  and  $n_p$  are the refractive indices of the water and dry polymer, respectively.

### 2.5.2 Contact angle measurement

The water static contact angles on the polyelectrolyte multilayer films were determined at the liquid–solid–air interface with the sessile drop method at room temperature. An Attension Theta Lite tensiometer from Biolin Scientific was utilized to conduct the measurement. A water droplet with a volume of 1  $\mu$ L was dropped by a Hamilton syringe onto the sample surface, and a CCD camera was used to record images of the water droplet profile. The contact angles after 5 seconds were then determined by fitting the Young–Laplace equation to the water droplet profile. A mean value of the left and right contact angles is reported as the static contact angle of the measured surface.



1. Cross-linking with EDC/NHS 2. Amine quenching with EDC/NHS/AcOH 3. Surface deprotection with TFA/DCM

**Scheme 1** Chemical structures of the polyelectrolytes and modification reagents used and the schematic illustration for the fabrication of the PMAA/PAMA PEM film with chemically modifiable amine outer layer

### **3** Results and Discussion

### 3.1 LbL assembly of PAMA/PMAA

The LbL assembly of PMAA and PAMA was monitored in-situ with simultaneous QCM-D and ellipsometry. Figure 1a presents the shifts in the 3rd overtone of the resonance frequency (F) and dissipation factor (D) of the quartz crystal sensor, resulting from the LbL assembly of the PMAA/PAMA multilayered film. The odd and even numbers of layers refer to the deposition of PMAA and PAMA layers, respectively, while the final layer (layer 14) denotes the deposition of the PAMA-co-PBocAMA copolymer. The decrease in frequency and increase in dissipation indicates continuous mass deposition on the substrate. The overall shifts in frequency and dissipation for seven bilayers are approximately -220 Hz and  $1 \times 10^{-6}$ , respectively. This provides a notably small  $\Delta D/\Delta F$  ratio of around 0.0045  $\times$  10<sup>-6</sup> Hz<sup>-1</sup> which suggests a highly rigid film structure with low water content.[36] It has to be noted that the deposition of PAMA-co-PBocAMA (last layer) shows a comparable frequency shift but a relatively larger dissipation shift compared to those of PAMA depositions. This can suggest that the outermost PAMA-co-PBocAMA layer adopts a more coil-like and less elongated conformation compared to the PAMA layers, which can be attributed to the relatively lower charge density (i.e.,  $\sim 60\%$  protected groups) of PAMA-co-PBocAMA. To further test the difference between a fully deprotected PAMA and partially protected PAMA-co-PBocAMA final layer, the contact angle of the film was measured at layer 12 (PAMA as the outer layer, Figure 1b) and layer 14 (PAMA-co-PBocAMA as the outer layer, Figure 1c). A clear variation in water contact angle between layer 12  $(25 \pm 1^{\circ})$  and layer 14  $(63 \pm 2^{\circ})$  is observed, which can be attributed to the relatively low charge density of PAMA-co-PBocAMA and the high hydrophobicity of the Boc protection groups.



**Figure 1** LbL assembly of PAMA and PMAA, with PAMA-co-PBocAMA as the final layer at pH 4.4 citric acid/phosphate buffer: a) Frequency (blue) and dissipation (red) shifts throughout the LbL process, with odd layers of PMAA and even layers of PAMA (PAMA-co-BocAMA as the final layer); b) Contact angle measured at layer 12, with PAMA as the final layer; c) Contact angle measured at layer 14, with PBocAMA as the final layer; d) Film thickness growth along with the PAMA/PMAA layer deposition obtained from QCM-D and spectroscopic ellipsometry

The film thickness was estimated using both Sauerbrey and viscoelastic Voigt models (Figure 1d). A close match between both models is found, which further confirms the rigid nature of the film.[36] The acoustic thickness of the film (14 layers) is then estimated to be around 35 nm. In addition, the thickness versus the number of layers demonstrates a closely linear trend (~ 5 nm per each bilayer), which indicates a linear growth mechanism. As reported in the literature, a linear growth mechanism is observed when the deposited polyelectrolytes cannot freely diffuse through the film, which seems to be the case herein. [37–39] Hence, we can assume that the deposited PAMA-*co*-PBocAMA has limited freedom to diffuse within the film and is confined to the film surface. This confined PAMA-*co*-PBocAMA distribution is important for our work, which requires a distribution of the modifiable amino groups predominantly on the outer layer of the PEM film.

The layer growth was simultaneously monitored with spectroscopic ellipsometry. The optical thickness of the film from the ellipsometry measurement is around 31 nm (Figure 1d), which is close (yet slightly smaller) to the estimated value from QCM-D. Besides, the estimated water content of the film (obtained from the BEMA) is approximately 21%. The relatively low water content is in accordance with the notably small QCM-D dissipation shifts. Such a compact structure of the PEM film might be attributed to a relatively high charge density of the polyelectrolytes and consequently a strong complexation between the PMAA and PAMA polymer chains.

## 3.2 Cross-linking and quenching of amino groups by EDC/NHS coupling

PEM films comprising weak polyelectrolytes are subject to disintegration towards drastic pH variation, due to a charge imbalance resulted from the protonation/deprotonation upon pH shift. To stabilize the prepared PMAA/PAMA(PAMA-*co*-PBocAMA) PEM film, the amino groups, and the carboxyl groups in the film were cross-linked utilizing EDC/NHS coupling chemistry.[40] Next, a pH cycle experiment was performed to examine the pH stability of the film. Figure 2 represents the QCM-D frequency and dissipation data (panel a), as well as the estimated optical thickness/water content (panel b) of the film at different pH values. Decreasing the pH from 4.5 to 2.5 results in an increment in dissipation and decrement in frequency, which together imply swelling of the film. Similarly, the estimated optical thickness roughly increases from 31 nm to 52 nm accompanied by a gain in the water content from 20 % to 50%. Increasing the pH back to 4.5 results in a shrinkage of the film yet a structural hysteresis is found. Accordingly, a slightly more swollen film is obtained after the first pH cycle, evidenced by a smaller frequency, larger dissipation, larger optical thickness, and larger water content. It is speculated that the strong PMAA/PAMA complexation within the film was disrupted at pH 2.5 and was only partially

reformed upon the pH returning to the initial value of 4.5. Nevertheless, the subsequent pH cycles exhibit a reversible swelling-shrinking process with minor hysteresis, indicating enhanced stability of the cross-linked film under acidic conditions. The subsequent pH cycles between 4.5 to 9 also demonstrate reversible swelling/shrinkage of the film under alkaline condition.



*Figure 2 pH-responsiveness of the film after cross-linking with EDC/NHS: a) QCM-D Frequency and dissipation and b) Optical thickness and water content (obtained from ellipsometry) following the pH oscillation test between pH 4.5 and 2, pH 4.5 and 9.* 

The swollen nature of the film at pH 2.5 can be attributed to the net positive charge of the film, which originates from the protonation of the excess amino groups. The unreacted amino groups

within the bulk film are not desirable for our work since we aim to prepare a film with amino groups mostly located in the outermost layer. Therefore, a previously described amine quenching process was conducted to effectively reduce the amine content in the film interior.[29] To do so, a solution of EDC/NHS, together with acetic acid, was flowed over the surface repeatedly for four consecutive cycles. As a result, acetic acid molecules will bind to the amino groups within the film transferring them into amide groups that are no longer reactive towards the later modification.

We examined the pH-responsiveness of the film under acidic conditions (after each quenching cycle) to test the efficiency of the amine elimination process. As is shown in the supporting information (Figure S5), the frequency and dissipation shifts associated with changing the pH from 4.5 to 2.5 both decrease in magnitude with the quenching process duration, indicating a decrease in the amine content following the process. Figure 3 shows the pH-responsiveness of the crosslinked PEM film after the amine quenching process. Regarding the pH cycles between 4.5 and 2.5, it is evident that the shifts in QCM-D frequency, as well as in the optical thickness and water content of the film, are significantly attenuated. This indicates that the population of the amino groups, which can produce film swelling under acidic conditions, is decreased. It can however be observed that there remains a minor responsive behavior of the film towards acidic pH (a minor frequency shift and a relatively large dissipation shift), indicating a small number of unquenched amino groups in the bulk film. Nevertheless, the effect is not considered major given the strong attenuation observed in frequency shift, optical thickness change, and water content change upon pH decrease. In contrast to the attenuated responsiveness to acidic conditions, the amine quenching process has a minor effect on the pH-responsiveness of the film under alkaline conditions. Accordingly, regarding the pH cycles between 4.5 to 9, similar swelling characteristics (compared to the film before amine quenching) are obtained in terms of the QCM-D shifts and variations in the optical thickness/water content.



*Figure 3 pH-responsiveness of the film after amine quenching: a) QCM-D Frequency and dissipation and b) Optical thickness and water content (obtained from ellipsometry) following the pH oscillation test between pH 4.5 and 2, pH 4.5 and 9* 

### 3.3 Surface deprotection and modification of PMAA/PAMA PEM film

Next, we discuss how deprotection of Boc-protected amino groups with TFA can modify the outermost PAMA-*co*-PBocAMA layer. Figure 4 presents the contact angles of the PEM film as a function of the duration of TFA/DCM treatment. Accordingly, the initial contact angle (before deprotection) of the film was around  $60 \pm 3^{\circ}$ . Following the deprotection process, a decrement in

the contact angle was observed, which can be attributed to the transform of the hydrophobic Boc groups to the hydrophilic amino groups on the surface. The contact angle value seems to reach a plateau of around 37° after 60 min of TFA/DCM treatment, which suggests a major removal of the Boc protection groups at 1h. Therefore with 1h of deprotection under the TFA/DCM condition, we successfully prepared the desired PEM film with amino groups situated in the outermost layer.



**Figure 4** Contact angle measured along the deprotection process, with a time interval of 30 min Now having these amino groups in the outermost layer, one method to modify the interfacial chemical composition is to bind carboxyl groups through carbodiimide chemistry. Hence, we chose two molecules functionalized with carboxyl groups, one hydrophilic (m-PEG3-COOH) and the other hydrophobic (undecanoic acid), to modify the surface hydrophilicity. Figure 5 compares the surface contact angle of the modified surfaces. Modification with m-PEG3-COOH modification led to a surface contact angle to  $55 \pm 2^{\circ}$ , which is in accordance with the typical literature value of a surface modified by PEG units.[41,42] Contrarily, modification of the film
surface with undecanoic acid resulted in an increment in the contact angle from  $37 \pm 2^{\circ}$  to  $78 \pm 3^{\circ}$ , which can be explained Nevertheless, despite a relatively high contact angle of  $78 \pm 3^{\circ}$ , it shall be noted that the surface cannot be regarded as hydrophobic. We speculate the reason to be a relatively low grafting density since only 63% of the PAMA-*co*-PBocAMA copolymer units were protected in and are feasible for surface grafting.



*Figure 5* Contact angles measured before and after surface modification with undecanoic acid and *m-PEG3-COOH* with DCC as cross-linking agent

We also tested the pH-responsive behavior of the modified films to check if the surface modification process affects the pH-responsiveness of the film. Figure 6 demonstrates the QCM-D frequency and dissipation shifts resulting from the pH cycles. The observed pH-responsive pattern is similar to that after amine quenching, indicating a minimum effect of the surface modification on the pH-responsiveness of the film. Therefore, the method raised in this work can be regarded as a versatile approach to the preparation of a cross-linked and pH-responsive PEM network with tunable interfacial properties.



*Figure 6* Frequency and dissipation shifts corresponding to a pH cycle from pH 4.5 to pH 2.5, and pH 4.5 to pH 9, after surface modification with a) undecanoic acid, b) m-PEG3-COOH

# 4 Conclusion

In summary, we have demonstrated a simple and versatile approach to fabricate a pH-responsive PEM film with a chemically modifiable outer layer. The prepared PMAA/PAMA multilayered film demonstrated pH-responsiveness originated from the amine and carboxyl group components in the film, evidenced with QCM-D and ellipsometry. We quenched the amino groups inside the film and removed the Boc protection groups on the outer layer. Correspondingly a film was obtained that comprised primary amino groups selectively in the outer layer and is feasible to chemical modification with carbodiimide chemistry. As proof of concept, the film outer layer was modified with m-PEG3-COOH and undecanoic acid. The PEGylated surface showed a contact angle of approximately  $55 \pm 2^{\circ}$ , while the surface modified with undecanoic acid exhibits higher hydrophobicity with a contact angle of  $78 \pm 3^{\circ}$ . Moreover, a similar pH-responsiveness of the two modified PEM films was observed, suggesting a minimum effect of the surface modification to the bulk property of the film. We believe this work provides a new methodology in fine-tuning of the outer-surface property of a PEM film and enrich our toolkit in surface functionalization for a wide range of applications.

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Supporting Information for

# A cross-linked and pH-responsive Polyelectrolyte Multilayer Film with Tuneable Interfacial Properties

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# **S1** Polymer Synthesis and Characterization

#### S1.1 Synthesis of PMAA

For the synthesis of PMAA, EBiB (initiator, 19.5 mg, 0.1 mmol), *tert*-butyl methacrylate (monomer, 1.42g, 10 mmol), PMDETA (ligand, 17.3 mg, 0.1 mmol), and copper(II) chloride (2.7 mg, 0.02 mmol) were dissolved in 2 ml isopropanol. Dimethylformamide (0.1 ml) was added to the reaction mixture as an internal standard for subsequent calculation of the monomer conversion. The reaction mixture was bubbled with argon for 30 min, after which the prewashed Cu(I) chloride (1.23 mg, 0.0125 mmol) was added. The polymerization was triggered by immersing the reaction flask into a 50 °C water bath. After 18 hours, the reaction was quenched by exposure to air. A small volume of the solution (0.1 ml) was collected for <sup>1</sup>H NMR measurement to determine the reaction conversion. The reaction mixture was afterward poured into a 20 ml 50% (v/v) methol/water solution to yield a white precipitate. The t-butyl protected PtBMAA polymer was filtered under vacuum, washed with 50% (v/v) methol/water solution, and dried under vacuum before further use.

In the deprotection process, the obtained PtBMAA polymer was dissolved in 4 ml dichloromethane followed by the addition of 0.5 ml trifluoroacetic acid. The reaction mixture was stirred at room temperature for 3 hours, after which dichloromethane was removed by evaporation. The white polymer powder was redissolved in 5 ml of water, and purified by dialysis and subsequent lyophilization. A <sup>1</sup>H NMR spectrum was obtained to confirm the complete removal of t-butyl protecting group (Figure S1). The molecular weight and polydispersity of PMAA were determined by asymmetric flow field flow fractionation. (Figure S2)



*Figure S1* <sup>1</sup>*H NMR of PMAA, with t-butyl protection group (bottom) and after deprotection (top)* 



*Figure S2* AF4 chromatogram of PAMA (red) and PMAA (blue)

#### S1.2 Synthesis of PAMA and PAMA-co-PBocAMA

The PAMA polymer was synthesized following a similar protection-deprotection protocol. To synthesize the Boc protected PBocAMA polymer, EBiB (initiator, 5.9 mg, 0.03 mmol), (2-Boc-amino)ethyl methacrylate (monomer, 687.8 mg, 3 mmol), PMDETA (ligand, 5.2 mg, 0.03 mmol), and copper(II) chloride (0.8 mg, 0.006 mmol) were dissolved in 2 ml isopropanol. A similar reaction procedure as in the synthesis of PMAA was conducted to start the polymerization. After the polymerization, the reaction mixture was passed through a neutral alumina column to remove the catalyst residue. The collected polymer solution was diluted into 20 ml of water to precipitate the PBocAMA polymer.

The PBocAMA was deprotected to provide either the PAMA homopolymer or the partially protected PAMA-*co*-PBocAMA copolymer, depending on the deprotection time. In order to completely remove the Boc protecting group and obtain the PAMA polymer, 100 mg PtBocAMA polymer was dissolved in 4 ml dichloromethane, after which 0.5 ml trifluoroacetic acid was added. The mixture was allowed to react for 3h, to thoroughly remove the Boc protecting groups, which was confirmed by the complete disappearance of the Boc peak observed from <sup>1</sup>H NMR spectrum (Figure S3). After deprotection, dichloromethane was removed via evaporation, and the raw polymer was dissolved in water and purified with dialysis and subsequent lyophilization. The molecular weight and PDI were measured by AF4. (Figure S2)



*Figure S3* <sup>1</sup>*H NMR spectrum of PAMA, with Boc protection group (bottom) and after deprotection (top)* 

In order to prepare the partially protected PAMA-*co*-PBocAMA, the PBocAMA polymer was deprotected with the same reaction condition. However, the deprotection process was terminated by evaporation of solvent 10 min after TFA was added. The remained PAMA-*co*-PBocAMA was dissolved in water and purified by dialysis and lyophilization.

The deprotection ratio of the PAMA-*co*-PBocAMA polymer was determined via <sup>1</sup>H NMR spectroscopy. (Figure S4) The ratio of integrals of the peak for Boc methyl groups (a, 1.5ppm) and methyl groups on the polymer methacrylate backbone (b, 0.8 - 1.3 ppm) was compared for the estimation. The ratio of remained protection Boc groups r is calculated as follows.

$$r = \frac{I_a/I_b}{3} = \frac{1.9/1}{3} \approx 0.63$$

Where I<sub>a</sub> and I<sub>b</sub> denote the integral of the peaks a and b, respectively.



*Figure S4* <sup>1</sup>*H NMR spectrum of the partially deprotected PAMA-co-PBocAMA copolymer* 

## S1.3 Polymer characterization

*NMR* A Brucker 400 MHz NMR spectrometer was utilized and <sup>1</sup>H NMR spectra were obtained with deuterated chloroform as the solvent. To determine the monomer conversion of each polymerization, samples of the reaction mixture were collected before and after the polymerization process, and corresponding <sup>1</sup>H NMR spectra were obtained. The integral ratios of  $\delta$  6 ppm (monomer double bond (vinyl))/ $\delta$  7.9 ppm (DMF) before (r<sub>1</sub>) and after (r<sub>2</sub>) the polymerization were used to calculate the monomer conversion (c) via  $c = 1 - r_2/r_1$ .

*AF4* To determine the number average molecular weight  $(M_n)$ , weight average molecular weight  $(M_w)$ , and polydispersity index (PDI) of the synthesized PMAA and PAMA, AF4 was performed

using a Wyatt Eclipse instrument with UV (Agilent 1230 infinity, Agilent), refractive index (Optilab rex, 633 nm, Wyatt) and multi-angle light scattering (MALS) (Dawn Heleos-II, 662 nm, Wyatt) detectors. A frit-inlet channel equipped with a PES membrane (MWCO 5 kDa, Millipore) and a W350 spacer was used as the separation channel. The samples were analyzed with a constant detector flow of 0.5 mL/min, and a cross-flow that decreased exponentially from 3 mL/min to 0 mL/min in 20 min. A 50 mM PBS buffer at pH 7.4 was prepared and filtered with a 0.1 µm membrane (Millipore) was used as the eluent. The samples were dissolved in the PBS buffer with a concentration of 5 mg/ml, and the injection volume was set to be 50  $\mu$ L. The chromatograms of the polymers were obtained from the RI detector and the molecular weight was determined by MALS. (Figure S4) The refractive index increment (dn/dc) values for PAMA and PMAA were obtained from literature<sup>29,30</sup> and the molecular weights were determined by employing a Debye analysis in the Astra software (Wyatt, version 7.1.3.15).<sup>31</sup> The M<sub>n</sub>, M<sub>w</sub>, and PDI of PAMA and PMAA were obtained from the measurement. The M<sub>n</sub> value of the copolymer PAMA-co-PBocAMA was calculated based on the Mn value of PAMA obtained from AF4 measurement, and the deprotection ratio obtained from <sup>1</sup>H NMR. The PDI value of the copolymer PAMA-co-PBocAMA is considered the same as that of PAMA since they were obtained from the same PBocAMA polymer.

# S2 pH Test with QCM-D after Each Amine Quenching Cycle



*Figure S5 AF4 Frequency and dissipation shifts upon pH change from 4.5 to 2.5 after each amine quenching process* 

Overall four amine quenching processes were performed on the cross-linked PAMA/PMAA PEM film to minimize the number of free amino groups. The first two quenching processes were 2h each, while the last two took a longer duration of 12h each. After each quenching, a pH 2.5 solution was flowed into the QCM-D cell to examine the pH triggered swellability of the film under acidic condition. The frequency and dissipation shifts upon each pH test were shown in Figure S5.