Addressable Macromolecular Architectures:

Towards stimuli promoted motion at the nanoscale

Edit Kutnyánszky

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ADDRESSABLE MACROMOLECULAR
ARCHITECTURES:
TOWARDS STIMULI PROMOTED MOTION AT THE
NANOSCALE

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*'Our greatest weakness lies in giving up.
The most certain way to succeed is always
to try just one more time.'*
Thomas A. Edison

*This Thesis is dedicated to my Sister
Csominak*

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Chapter 1

Towards stimuli promoted motion at the nanoscale

1.1 Introduction

Macromolecular motors are among the most fascinating architectures built by Nature.¹ These complex, protein based systems undergo dramatic conformation changes in order to carry out their task. They are capable of linear or rotary motion to directionally transport cargo in biological systems.² The desire, and even more, the need to construct similarly elegant systems based on (synthetic) macromolecules provide continuous inspiration as well as offer a challenging task for scientists.³ In Nature, molecular machines operate at interfaces, therefore it is a logical step in a synthetic environment to construct surface confined systems⁴. The most outstanding examples feature small organic molecules that turn unidirectionally 360° driven by light irradiation,⁵ as well as nanocars fueled by electrical energy⁶ based on chiral organic molecules. A major difficulty is to achieve directional translational motion on the molecular level,^{7,8} while random motion on the surface is 'easily' achieved by translation of macromolecules on surfaces guided by the structural lattice of the surface.^{9–12}

Stimulus responsive polymers hold the promise of becoming essential parts of functional devices and systems.¹³ These polymers recognize a stimulus as a signal and subsequently alter their chain conformation in a direct response accompanied by variations in their physical properties, such as stiffness, optical absorbance, electronic structure or polarizability. Stimulus responsiveness is present in all levels of ensembles including single chains, surface grafted polymer brushes, macromolecular bottlebrushes and core-shell micelles. The properties of a macromolecular ensemble that consists of complex molecular architectures can be derived from the attributes of the single chain constituents. In these structures, addressability results from the sensitivity of incorporated polymer chains to variations in temperature, electric or magnetic field strength, pH or ionic strength. To utilize stimulus responsive behavior and construct 'functioning' molecular nanosystems such as molecular crawlers or motors, an external stimulus, as a signal, should be applied directly to a specific polymer chain, and be used for controlling their chain conformation and thus physical properties.

Macromolecules responsive to multiple types of stimuli are attracting increasing interest.¹⁴ Such molecules are often constructed from several building blocks where each constituent responds to a different stimulus.

Although redox responsive polymers have received relatively little attention,¹³ redox is one of the very few stimuli that allows one to directly address single molecules.

Synthetic polymers containing metal centers (metallopolymers¹⁵) can be addressed by oxidation or reduction in appropriate solvent environment. One of the most promising redox active polymers for practical applications as 'smart' materials belongs to the group of poly(ferrocenylsilanes) (PFSs). Due to the presence of ferrocene in the main chain, PFSs are electrochemically active, and can be reversibly oxidized/reduced.¹⁶

In order to understand the behavior of stimulus responsive polymer systems, it is fundamentally essential to explore their behavior on the single chain level. To this end, *i.e.* to visualize changes in molecular structures and molecular scale motion upon a stimulus, a powerful tool is needed. The advent of probe microscopy techniques, especially atomic force microscopy (AFM),¹⁷ enables one to observe changes in properties of macromolecular systems in real time and characterize and manipulate¹⁸ surface confined polymer structures at the nanoscale. AFM has been used with great success 'beyond imaging'. Probing nanoscale properties,¹⁹ measuring molecular forces²⁰ and manipulating nanostructures including molecular construction, single molecule delivery and molecular 'cut and paste'²¹ are among the most interesting examples of AFM applications.

1.2 Concept of this Thesis

The concept of the research described in this Thesis is centered around the design, construction and evaluation of stimulus responsive polymeric systems at different levels of architectural complexity, from a one dimensional single chain to three dimensional side chain grafted copolymers.

Chapter 2 provides an overview of stimulus responsive polymer architectures and summarizes the relevance of such materials in the area of materials science. The first part of the Chapter describes chemical methods to construct addressable macromolecules. Physical properties of such polymer structures are also discussed. Furthermore relevant tools to study the responsive behavior of these macromolecular architectures such as dynamic light scattering and atomic force microscopy are introduced. The second part of the Chapter focuses on application of atomic force microscopy for characterization of polymers at the nanoscale.

For the characterization of grafted polymer monolayers (brushes) we employed AFM to investigate nanomechanical properties of end grafted polymer layers. In **Chapter 3** as a representative example, a zwitterionic poly(sulfobetaine methacrylate) (PSBMA) brush, grafted from a planar Si surface and a poly(methacrylic acid) (PMAA) brush, grown on a colloidal AFM probe were studied. Force-distance curves were obtained and the grafting density according to the theory of de Gennes²² was determined. The apparent value of the Young's modulus, analyzed by the Hertz model,²³ was also determined.

AFM based single molecule force spectroscopy (AFM-SMFS) is often used for the detection and mechanical characterization of single molecules under environmentally

controlled conditions. In **Chapter 4** as a next step to observe single chain responsive properties, the molecular stretching behavior of temperature responsive poly(*N*-isopropylacrylamide) (PNIPAM) chains was studied by AFM-SMFS. Force-extension curves obtained in water below and above the lower critical solution temperature (LCST), in the co-nonsolvent mixture water/methanol and in dimethyl sulfoxide (H-bond blocking) followed the same trajectory, regardless whether the chain was pulled from a collapsed or from a solvated state. This result indicates that for a single PNIPAM chain the formation of intrachain H-bonds in the precipitated state does not cause measurable chain stiffening at the single chain level.

For stimulus-propelled molecular crawlers we chose a system that exhibits dual responsive behavior, consisting of a PFS backbone and PNIPAM side chains (PFS-*g*-PNIPAM). In **Chapter 5** corresponding molecular bottlebrushes were discussed. These macromolecules were obtained by first forming the backbone, followed by side chain grafting. To prepare the dual stimuli responsive macromolecules a 'grafting to' as well as 'grafting from' process were used. The 'grafting to' method involved a Huisgen cycloaddition click reaction between an azide functionalized PFS backbone and alkyne end-functionalized PNIPAM chains.²⁴ In the 'grafting from' method, ATRP initiator moieties were connected as side groups to the PFS backbone to form and organometallic macroinitiator.

Three dimensional graft bottlebrushes can undergo a change in their size and/or shape in response to an external environmental change. The response to temperature of the PNIPAM side chains, and variation in the oxidation state of ferrocene in the backbone were studied in detail as a function of the structure of the bottlebrushes in **Chapter 6**. For all PFS-*g*-PNIPAM bottlebrushes, in aqueous solution, LCST behavior was observed, and T_{LCST} was determined to be 32 °C which is essentially identical to that of PNIPAM. As monitored by dynamic light scattering measurements, bottlebrushes undergo a reversible linear decrease in size over a wide temperature range, until the LCST is reached where either intra- or intermolecular collapse was observed. PFS-*g*-PNIPAM macromolecules deposited on a HOPG surface showed a response to electrochemical signals, resulting in a double wave cyclic voltammogram typical of PFS. The shape of the voltammogram changes when the temperature is increased above the LCST.

A grafting density gradient was obtained in the side chains by creating a composition gradient within the PFS backbone of functionalizable monomer units and nonfunctionalizable ones. **Chapter 7** describes the synthesis of the asymmetrical PFS-*g*-PNIPAM bottlebrushes. By controlling the feed ratio of the two applied monomers, a continuous composition decay of one of them was achieved. Stimuli responsive behaviour of the gradient bottlebrush was tested through its electrochemical response. In organic electrolyte, a typical double wave cyclic voltammogram was recorded, while in aqueous environment the shape of the voltammograms was highly influenced by the inhomogeneous distribution of the water soluble PNIPAM along the backbone and further changed when the temperature was raised above the LCST.

In principle, during oxidation-reduction cycles, the PFS chain periodically extends

and retracts along the chain direction, while the phase transition of PNIPAM manifests itself in an adhesion change towards the underlying surface. Therefore, a sequential variation in the externally applied stimuli should result in directed motion of the molecule on a suitable surface. In **Chapter 8** we summarize principles of the molecular movement of a nanocrawler and discuss how characteristics of the underlying surface can in principle be tuned to assist in molecular motion.

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Chapter 2

Addressable polymeric nanostructures: From single chains to side chain grafted copolymer based molecular architectures and their characterization by AFM*

This Chapter provides an overview of stimulus responsive polymer architectures in the literature and summarizes the relevance of them in the area of materials science. The first part of this Chapter describes chemical methods to construct addressable macromolecules, while in the second part some physical properties of these addressable polymer structures are discussed. In particular dynamic light scattering and atomic force microscopy based force measurements will be discussed in detail as tools to study the responsive behavior of these macromolecular architectures.

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2.1 Introduction

Stimulus responsive polymers

Addressable polymer structures play an increasingly important role in a variety of areas, such as surface engineering, drug delivery and micro/nanofluidics.¹

Addressability of these structures results from the sensitivity of incorporated polymer chains to (often small) changes in their environment. There are many different stimuli to control the response of these polymer systems. These stimuli can be classified to have either a physical or chemical origin. Chemical stimuli, *e.g.* pH, ionic factors and chemical agents, change the interactions between polymer chains or polymer chains and solvent(s) at the molecular level. Physical stimuli, *e.g.* temperature, electric or magnetic fields and mechanical stress, affect the level of various energy sources and alter the molecular interactions at critical onset points.²

Temperature is the most widely used stimulus in environmentally responsive polymer systems. Temperature variation is not only relatively easy to control, but also readily implemented in applications for instance in the engineering or biomedical field. Poly(N-substituted acrylamides) are representative for the group of temperature responsive polymers, which have a lower critical solution temperature (LCST). This transition temperature is defined as the critical temperature at which a polymer solution undergoes phase transition from a soluble to an insoluble state. One of the most studied stimulus responsive synthetic polymers, is poly(N-isopropylacrylamide) (PNIPAM)³ (Figure 2.1). Since its LCST temperature ($T_{LCST} = 32\text{ }^{\circ}\text{C}$, in aqueous media) is close to the body temperature, PNIPAM has attracted significant interest for utilization in biomedical applications.⁴ A detailed discussion of the LCST behavior of PNIPAM under mechanical stress, at the single chain level is provided in Chapter 4. There are several other temperature responsive polymers known with different T_{LCST} values. Poly (ethylene glycol)(PEG) ($T_{LCST} = 45\text{ }^{\circ}\text{C}$) and its derivatives are well known for their biocompatibility, and therefore a preferred material of choice in medical applications.² Poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) is also an often used polymer in medical applications, however it has a somewhat higher transition temperature compared to the previous mentioned polymers ($T_{LCST} = 50\text{ }^{\circ}\text{C}$).²

Analogous to temperature responsiveness, medium-change driven responsive behavior can be triggered by *e.g.* variations in concentration, solvent quality or solvent pH (for polyionic chains). pH responsive systems have drawn attention as chemical actuators in responsive hydrogels.^{5,6} For example, poly(acrylic acid) (PAA) and poly(methacrylic acid) (PMAA) (Figure 2.1) become negatively charged by deprotonation at $\text{pH} > \text{pK}_a$. Repulsion of the charges along the polymer chain force the coiled molecules to obtain a stretched conformation upon changing the pH from below pK_a to above pK_a and *vica versa*. We note that some temperature responsive polymers *e.g.* PNIPAM and PDMAEMA also exhibit pH responsive behavior.

Synthetic polymers containing metal atoms (metallopolymers),⁷ can be addressed by oxidation or reduction in an appropriate solvent environment.^{8–10} For example,

Figure 2.1: The chemical structures of the stimuli responsive polymers; (a) and (b) temperature responsive: PNIPAM and PEG, respectively (c) and (d) pH responsive PMAA and PAA, respectively (e) pH and temperature responsive PDMAEMA, (f) redox responsive PFS, (g) light sensitive azobenzene moiety.

wormlike motion was achieved in a metallopolymeric hydrogel as a result of the successful incorporation of a Ruthenium complex in the polymer.^{10–12}

One of the most promising redox active polymers are the group of poly(ferrocenylsilanes) (PFSs), featuring a polymer chain consisting of ferrocenyl units connected by substituted silanes (Figure 2.1). Due to the presence of ferrocene in the main chain, PFSs are electrochemically active and can be reversibly oxidized and reduced.^{13,14} Further unique and highly interesting properties of PFSs concerning applications include a high etch resistivity in reactive ion etching environments (with relevance to lithography),¹⁵ catalytic activity, sensing, electrochromic materials,^{16,17} etc. The choice of the substituents on the silicon atom, *i.e.* the primary chemical structure of the chain, controls the physical properties of the polymer. Symmetric as well as asymmetric substitution is possible with, *e.g.* methyl, ethyl, phenyl, etc. Furthermore when ionic substituent groups are attached -to the silicon atom- the PFS becomes water soluble.¹⁸

A wide variety of photo responsive polymers have been studied with a great potential for utilization in light-controllable functional materials.^{19–21} The conformational changes of the polymers induced by light irradiation enables one to tailor their physical and chemical properties.²² The most widely studied photoresponsive polymeric system incorporates azobenzene moieties (Figure 2.1) either in the side chains or in the main chains.^{22–24} Azobenzene groups are known to undergo a reversible isomerization from *trans*-to *cis*-configuration upon UV-VIS light irradiation, providing a possibility for the design of smart architectures exploiting their picosecond reaction times to the external stimuli.

Successful applications of single stimulus responsive polymers led to an increased

interest in macromolecules that are responsive to multiple types of stimuli.²⁵ In general, these macromolecules consist of two or more building blocks that are separately responsive to a different stimulus.

In addition, coupling between responses due to sequentially applied stimuli may result in new, fascinating properties of such macromolecular systems.^{26–29} Hence utilization of stimulus responsive molecules provide a broad range of applications. Construction of two or three dimensional architectures allows one to design for instance responsive biointerfaces that resemble natural surfaces, controlled drug-delivery and release systems,² composite materials that actuate and mimic the action of muscles³⁰ and thin films or particles that are capable of sensing very small concentrations of analytes.³¹ The following paragraph will provide a brief introduction to common synthetic strategies used to prepare responsive architectures.

2.2 Polymeric structures

Single polymer chains consist of monomeric units that are covalently bound to each other through a polymerization reaction. These macromolecules usually represent organic compounds, containing carbon, hydrogen, oxygen, nitrogen and halogen atoms but can also incorporate inorganic, metal atoms such as Fe. Polymerization methods usually result in a mixture of macromolecules with various molar masses and averaged degree of polymerization (N , amount of monomeric units in the chain). Hence characterization of the molar mass and its distribution is necessary when studying or utilizing polymers. This is achieved by determination of the number average (M_n) and weight average (M_w) masses. Polydispersity (PDI) of the polymer is defined as the ratio of the weight and number average molar masses ($PDI = M_w/M_n > 1$). For a uniform behavior of the polymer system it is desirable to be close to $PDI = 1$.³²

Living, controlled radical polymerizations allow one to synthesise well-defined macromolecular architectures with functional groups. Atom transfer radical polymerization (ATRP), reversible addition-fragmentation chain transfer (RAFT) polymerization, single-electron transfer living radical polymerization (SET-LRP) and nitroxide-mediated polymerization (NMP)³³ afford well-defined polymers with a controlled molar mass, low polydispersity. Monomers that incorporate inorganic atoms usually can not be polymerized via the above mentioned controlled radical polymerization methods. For these monomers, anionic -, metal catalysed polymerization or polycondensation reactions can be used, however these methods usually result in a more polydisperse product.³⁴

Since, it is our interest to construct stimulus responsive architectures, exploiting several types of polymerization methods is required. Hence the used methods will be introduced and discussed in details.

2.2.1 General polymerization processes

Reversible addition-fragmentation chain transfer polymerization

Reversible addition-fragmentation chain transfer polymerization (RAFT) proceeds via a degenerative transfer process and relies on the use of compounds employed as chain-transfer (RAFT-CTA) agents. These agents are organic compounds possessing a thiocarbonylthio moiety. The generally accepted mechanism for a RAFT polymerization consists of five steps: (i) initiation, (ii) initial equilibrium, (iii) re-initiation, (iv) main equilibrium and finally (v) the termination step, as is shown in Figure 2.2.

Figure 2.2: Generally accepted mechanism for RAFT polymerization. Following initiation (i), the radical reversibly adds onto the chain transfer agent 1 to form an intermediate radical 2, which can fragment to liberate a re-initiating group and form a new dormant chain 3 (ii). The new radical re-initiates the polymerization by the reaction on monomers (iii). The rapid establishment of this reversible addition-fragmentation equilibrium (iv) provides control over the molar mass and the molar mass distribution, although irreversible termination reactions still occur, mainly due to free radicals introduced initially to initiate polymerization (v). 1a, 1b and 1c: examples of the chain transfer agents: the R group initiates the growth of polymeric chains, and the Z group activates the thiocarbonyl bond towards radical addition and stabilizes the resultant adduct radical. [Reprinted by permission from Macmillan Publishers Ltd³⁵].

In the initiation step a radical is created, that further reacts with the RAFT-CTA (Figure 2.2: 1, 1a and 1b), establishing the first equilibrium of the initiator radical system. Due to the high reactivity of the C=S bond of the RAFT agent, radical addition is favored over the addition to any of the double bonds that are present in the monomer, leading the consumption of the CTA first. After re-initiation,

polymer chains grow by adding monomers, leading to the main equilibrium between the multiple radicals that are present in the system, while in the propagation step the species are capped with the thiocarbonylthio group. Due to the rapid interchange in the chain-transfer step, termination reactions are limited, although these reactions can occur via combination or disproportionation. The composition of the R and Z groups (Figure 2.2 1, 1a and 1b) are of critical importance to a successful RAFT polymerization, the R group initiates the growth of polymeric chains, and the Z group activates the thiocarbonyl bond towards radical addition and stabilizes the resultant adduct radical. The R group of a RAFT agent plays a role in the pre-equilibrium stage of the polymerization, it should be a better leaving group compared to the propagating radical and must efficiently re-initiate monomer as an expelled radical.

Atomic transfer radical polymerization

In atomic transfer radical polymerization (ATRP)^{36,37} alkyl halide molecule serves as an initiator RX (Figure 2.3). During the reaction there are so called activators, that are low-oxidation-state metal complexes Mt^zL_m (Mt^z represents the metal species in a given oxidation state z , L is a ligand), and deactivators, which are higher-oxidation-state metal complexes with coordinated halide ligands ($XMt^{z+1}L_m$). After activation of the initiator ($R\cdot$), the polymerization proceeds due to the reversible formation of propagating radicals ($Pn\cdot$). Polymer chains that can grow are present as dormant species (P_nX) in the ATRP equilibrium. ATRP is a catalytic process that is mediated, usually by redox-active transition metal complexes, such as the most commonly used Cu, (other metals can also be used e.g.: Ru, Fe, Mo, Os).

A limitation of ATRP is the requirement of a relatively large amount of catalyst, although the rate of the polymerization does not depend on the absolute catalyst concentration, but on the ratio of the activator and deactivator concentrations. Typically 0.1-1 mol% catalyst relative to the monomer is added to the reaction to avoid inevitable termination reactions, which results in the presence of a significant amount of residual metal in the final product. To overcome this problem, efforts were made to develop ATRP techniques that are operated at extremely low catalyst concentrations while keeping the activator/deactivator ratio constant. The developed initiation systems³⁸ utilize very small amounts of active catalyst and the deactivator that forms due to radical termination is constantly converted to activator through a redox process. Simple reducing agents such as ascorbic acid, sugars, tin(II) octanoate, or amines are used in the so called activators regenerated by electron transfer (ARGET-ATRP)³⁹ method and radical initiators in the initiators for continuous activator regeneration (ICAR-ATRP)³⁸ method.

The catalytic activity and selectivity is strongly ligand dependent (L_m). For Cu catalysed reactions, usually amine derivatives are used, that can coordinate Cu^I in a square tetrahedral or planar configuration (that is preferred by the ion). Acidic or amine pendent group containing monomers can also coordinate Cu ions, therefore it is essential that the ligand complexes the metal relatively strong, otherwise the

Figure 2.3: The reaction scheme for ATRP including the activator regeneration methods ICAR (initiators for continuous activator regeneration) - where the activator is regenerated using a radical initiator and ARGET (activators regenerated by electron transfer) - where reducing agents are used. P_n represents the polymer (with degree of polymerization n), Mt^z is the metal species in oxidation state z , L is a ligand and X is a halogen atom. Changes in the oxidation state are represented by changes in colour. The kinetic parameters k_{act} , k_{deact} , k_p and k_t represent the rate constants of activation, deactivation, propagation and termination respectively [Reprinted by permission from Macmillan Publishers Ltd³⁷].

reaction becomes uncontrolled.³⁶

Each of these controlled radical polymerization methods have certain advantages and limitations. Due to the development of the new methods in ATRP low catalyst concentrations are sufficient in contrast to RAFT that uses stoichiometric amounts of dithioesters as mediating agent. However RAFT polymerization is a more versatile process, as it is tolerant to a wide variety of reaction conditions and functionalities.³⁵ Several classes of monomers like styrene derivatives, acrylates and acrylamides, methacrylates, methacrylamides as well as vinyl esters can be polymerized by RAFT.⁴⁰ ATRP includes the more reactive (meth)acrylates, styrenes, acrylonitrile, but also the less reactive vinyl acetate and vinyl chloride.³⁶ However, ATRP of acidic monomers requires protection or neutralization of the monomer, while RAFT is incompatible with basic monomers and those with primary amino groups.

Ring-opening polymerization of [1]silaferrrocenophanes

Ring-opening polymerization (ROP) represents an important, well-established route to obtain organic polymers and is an increasingly successful method for the syn-

thesis of inorganic macromolecules.⁴¹ Synthesis of organometallic polymers from strained metallocenophanes can be achieved by ROP as the monomers are cyclic compounds. The general scheme is shown in Figure 2.4. The first ROP via thermal induction (TROP) of PFS was reported by Manners *et al.*⁴¹ The ring-strained [1]silaferrocenophane monomers can easily be polymerized through TROP,⁴² however, this method is not often applied because (i) the polymerization required a high boiling point solvent and (ii) the choice of the side groups of the Si atom is limited as compared to other methods. Nowadays the transition metal catalyzed (ROMP) and the living anionic ROP (LA-ROP) are preferred.

Figure 2.4: Ring-opening polymerization of ring strained sila[1]ferrocenophane via metal catalysis (ROMP) or living anionic (LA-ROP) routes.

The transition-metal catalysed ROP of ferrocenophanes is carried out in an appropriate solvent, usually tetrahydrofuran, at room temperature using Pt^I , Pt^{II} or Rh^I moieties as a catalyst, most likely in the form of heterogeneous metal colloids.⁴³ A sufficient molar mass control can be achieved when termination agents bearing a Si-H bond are used, by controlling the initial ratio of the monomer to the silane.⁴⁴ This method is tolerant to several side groups of the silicon atom, including chlorine or alkyl-Cl pendant groups, allowing a wide range of post modification—substitution reactions—along the polymer backbone.^{45–49} A disadvantage of this method is that polymers with relatively high polydispersity indices are produced, *i.e.* typical *PDI* values are 1.4–2.2.

Living anionic ROP is a well controlled way of synthesising PFSs. The polymerization proceeds under mild conditions using alkyllithium initiators. Since there are no significant termination or chain transfer reactions, LA-ROP allows one to prepare polyferrocenylsilanes with predictable molar masses and narrow molar mass distributions (even $\text{PDI}=1.2$ can be achieved). In addition anionic ROP can be induced by a photolytic reaction. The initiation occurs by irradiation of a monomer solution in the presence of an anionic initiator (sodium cyclopentadienylide) with UV-VIS light. However, this approach requires highly purified ferrocenophane monomers.⁵⁰ LA-ROP has been used with success to synthesize PFS block copolymers under mild conditions. There are several examples of block copolymers with blocks of different ferrocenylsilane derivative monomers prepared with a good control over the molar mass of each blocks as well as of block copolymers with polystyrene, poly(isobutylene) or poly(ethylene glycol).^{24,51–57}

Due to the availability of controlled polymerization routes for PFS monomers,

well-defined architectures with organic and inorganic blocks are available. Under mild conditions further substitution reactions can be carried out on the silicon pendant groups providing possibilities for the development of functional nanomaterials.^{16,24,49,58,59}

The above described polymerization methods are great contributions for the preparation of complex architectures.^{37,60} For instance, these methods can be applied to grow polymer chains from substrate attached initiators, obtaining polymer brushes or construct 3D compositions of multiple types of polymers *i.e.*: molecular bottlebrushes.

2.2.2 Polymer brushes, from surfaces to molecular bottlebrushes

Surface tethered polymer chains form thin layers, where the polymer chains are anchored by one end to various types of surfaces. Due to the often unique surface properties of these polymer brushes including tailorable wettability,⁶¹ adhesiveness,⁶² friction,⁶³ biocompatibility^{64,65} or anti-biofouling^{66–68} these films have received great attention in the past decades.⁶⁹

Figure 2.5: Schematic representation of the structural conformations of grafted polymer chains [Adapted from a reference⁷⁰].

As a structural definition of polymer brushes the grafting density (Γ) is the most commonly used characteristic. Γ expresses the amount of chains that occupies 1 nm^2 area on the surface ($\Gamma = 1/d^2$), where d is the distance between the polymer chains.⁷⁰ Sometimes Γ defined by the following equation⁷⁰:

$$\Gamma = \frac{h\rho N_A}{M_n} \quad (2.1)$$

where, h is the height of the brush, ρ is the bulk density of the brush composition, N_A is the Avogadro's number and M_n is the number average molar mass of the polymer chains. The morphology of polymer brushes depends on the grafting density (Figure 2.5). When the chains are well separated from each other, they adopt a 'pancake' conformation. If the distance is equivalent to the radius of gyration (R_g def. see section 2.4.1) of the polymer chains, the surface-anchored film enters the

mushroom regime. More densely packed chains are stretched out, forming polymer brushes. The transition point between two regimes is described by the reduced tethered density (Σ): $\Sigma = \Gamma \pi R_g$. When $\Sigma < 1$ a mushroom structure exists, while for $\Sigma > 1$ a polymer brush is found. In real brush systems Σ was found to be around 5.⁷⁰ Although it is one of the most important brush characteristics, still it is a notoriously difficult analytical question to determine its value. Another related challenge of brush characterization is the determination of the chain length and grafting density values of the brushes.⁷¹

To obtain surface grafted polymer layers, in general two methods are used. The polymer chains can either be 'pre-made' and 'grafted to' a surface⁷² or 'grafted from' a surface using various polymerization methods (see Figure 2.6). With the 'grafting to' method the maximum reachable grafting density is limited. The end-functionalized macromolecules are sequentially attached to an activated surface which results in the formation of a brush layer, however diffusion of the macromolecules towards the developing layer of tethered chains slows down, as the grafting density increases. Steric hindrance prevents the formation of dense brushes,

Figure 2.6: Schematic representation of brush formation methods. (a) 'Grafting to' leads a loose structure with a low grafting density, while by (b) 'grafting from' high density brushes are formed [Adapted from a reference⁷⁰].

in the 'grafting to' approach thus loops and tails are formed on the surface. Recent studies⁷³ however showed that by applying powerful coupling reactions such as the azide-alkyne click reaction, it is indeed possible to obtain high density polymer brushes via the 'grafting to' method. Besides the rapidly growing application of the click method,^{73–75} thiol-gold chemistry for modification of gold surfaces⁷⁶ as well as preparation of silane end modified polymers for silicon surface modification are well established methods for the 'grafting to' of polymer brush films.

The 'grafting from' method allows one to produce high density polymer brush films with well-defined polymeric chains via various polymerization techniques. In this case the substrate surface is functionalized by the attachment of initiator moieties,^{77,78}

forming an initiator 'monolayer'. The density of this layer is easily controlled which determines the grafting density of the brush layer. This way, brushes with certain chemical/physical properties^{71,79} as well as gradient surfaces are readily obtained.^{80,81} Due to high entropic forces between the chains, highly swollen/stretched brushes might be peeled off from the surface, therefore it is necessary to ensure a strong attachment of the initiator to the surface.⁸²

Polymer brushes consisting of stimulus responsive chains are ideal candidates for smart surfaces.^{6,83,84} In addition, combination of multiple types of polymers applied as mixed grafts of block copolymers enhances their capability to response to (several) external stimulus. For instance, these surfaces switch their surface properties that is manifested by a change in adhesion upon variation in temperature⁸⁵ or pH.^{86,87} Due to these interesting addressable property changes recently several promising applications have been demonstrated. For instance, responsive polymer brushes can serve as ion gating channels in nano/micropores^{88–91} or for preparation of nanosensor thin layers.^{8,92–94}

Molecular bottlebrushes

Molecular bottlebrushes (Bb) are graft copolymers consisting of a polymer backbone to which polymer side chains are attached. Since the first bottlebrush (prepared by ATRP) was reported in 1998, the area of molecular brushes has expanded rapidly. In [Figure 2.7](#) different branching topologies and 'chemical compositions' of molecular brushes are illustrated. The shown examples feature molecules with a longer backbone than their side chains. Therefore, these molecules are referred to as cylindrical bottlebrushes or molecular brushes. Conformation and physical properties are controlled by steric repulsion of the densely grafted side chains: the higher the grafting density, the lower the flexibility of the architecture which as a result presents rigid, rod like properties.^{95,96} By careful design of the composition, the grafting density along the backbone can be tuned i.e.: forming a gradient structure with the side chains.^{97,98}

Natural bottlebrush polymers, proteoglycans, fulfill a variety of biological functions in the human body.^{99,100} Early examples of molecular bottlebrushes featuring poly (*N*- butyl acrylate) side chains were already proposed as synthetic substituents for natural bottlebrush polymers. To further tune their properties towards biomedical applications stimulus responsive polymers have been introduced as building blocks.¹⁰¹ Development of bottlebrushes responsive to multiple stimuli is expected to yield novel, fascinating addressable materials.

Typical examples of stimulus responsive bottlebrushes possess poly(2-bromo-isobutyroxyethyl methacrylate) (PBIEM) or chloromethylated polystyrene (PS) backbones from which pH responsive or thermo responsive side chains were grafted by various polymerization methods. pH responsive chains include PAA, PMAA¹⁰² and PDMAEMA while temperature responsiveness is achieved by incorporating oligo- or poly(ethylene glycol) (PEG),¹⁰³ PDMAEMA or PNIPAM¹⁰⁴ grafts. In general

Figure 2.7: Branching topologies. The black and grey lines refers to a different chemical composition of the polymer chain [Reprinted from a reference⁹⁵ with permission from Elsevier. Copyright (2012)].

there are three methods for the synthesis of molecular brushes: 'grafting through' (polymerization of macromonomers), 'grafting to' (attachment of the side chains to the backbone), and 'grafting from' (growing the side chains from the backbone) (Figure 2.8). The 'grafting through' route involves the polymerization of macromonomers,¹⁰⁵ which are polymers with polymerizable end groups, 'through' their terminal functionality. This method allows the preparation of brushes with 100% grafting density, but because of the low concentration of polymerizable groups and the steric hindrance of side chains it is difficult to achieve molecular brushes with a high degree of polymerization, with a low polydispersity. In the 'grafting to'

Figure 2.8: Grafting methods to obtain bottlebrush molecules [Reprinted from a reference¹⁰¹ with permission from Elsevier. Copyright (2012)].

method,^{106,107} both backbone and side chains are prepared separately. This strategy involves the reaction of end-functional polymers with a polymer backbone precursor containing a complementary functionality on each monomer unit. However, the already attached side chains hinder the formation of high grafting density brushes. In the 'grafting from' method, a macroinitiator, consisting of a polymer backbone with initiation sites, is prepared and side chains are subsequently grown from the macroinitiator. The various controlled radical polymerization methods provide plenty of room for designing bottlebrushes with tailored structures, as these methods allow one to use a wide variety of monomers.^{108–111} In addition, ATRP has recently been used under biologically relevant environment for the grafting of biocompatible polymers from protein or polypeptide backbones.^{103,112,113}

2.2.3 Stimulus responsive properties of bottlebrushes

In response to an external environmental change such as a variation in pH,¹¹¹ temperature,¹¹¹ light,^{111,114,115} or magnetic field,¹¹⁶ Bb molecules undergo a change in their size and/or shape. In order to monitor these changes, commonly used observation techniques include dynamic light scattering (DLS) measurements^{106,114,115,117,118} and atomic force microscopy (AFM). Visualization of the molecules with AFM provides information on their structure¹¹⁹ and allows analysis of branching topologies^{108,120} and surface interactions.¹²¹ For a detailed description of these methods see Section 2.4.

In solution, the obtained shape is determined by the quality of the solvent for the polymers. In most applications it is preferred to be an aqueous environment, which can be a good solvent for the side chains but may be poor for the backbone. For example, thermoresponsive poly(propylene oxide)-*b*-poly(ethylene glycol) (PPO-*b*-PEG) or PEG-*b*-PPO core shell Bbs were prepared by anionic grafting from a PS backbone.¹¹⁸ The solubility of the Bb depended on the sequence of the PEG/PPO blocks. PPO, as an inner block, formed a core with the PS backbone, resulting

in intramolecular chain contraction/association upon heating. While the reversed order (PPO as an outer block) showed an expected LCST behavior, intermolecular aggregation and the measured hydrodynamic radius (R_h) (by DLS) increased from 14–24 nm to a finite aggregation size of 70 nm. In contrast to the first case no aggregation was observed, instead upon increasing the temperature the structure became more compact and less polydisperse, *i.e.* R_h steadily decreased from 22 to 18 nm.

Figure 2.9: Observation of intra- and inter-molecular aggregation of temperature responsive Bbs by DLS [Reprinted with permission from Elsevier [101,122](#)].

Matyjaszewski and coworkers used a PBIEM macroinitiator as the backbone for the 'grafting from' polymerization of methacrylates to produce poly(BIBM-*graft*-DMAEMA) and poly(BIBM-*graft*-(DMAEMA-*stat*-MMA) molecular brushes (Figure 2.9) by ATRP.¹²²

DLS studies were performed on aqueous solutions of these molecular brushes below and above the LCST. The measurement showed that incorporation of the hydrophobic MMA units into the PDMAEA chains reduced the apparent size of the brush molecules due to a decreased hydration and also lowered the LCST. In addition, an unusual concentration-dependent LCST behavior was observed. Due to the compact structure of molecular brushes, intramolecular collapse occurred when the average distance between the molecules is much larger than the hydrodynamic dimensions of the individual macromolecules. However, if the concentration of the solution of molecular brushes is increased to the level at which the separation distance is comparable with the brush hydrodynamic dimensions, intermolecular aggregation occurs, as typically observed for solutions of linear polymers. Therefore, the brushes

underwent a transition from extended cylinders to more compact morphologies with increasing temperature.

In a similar 'grafting from' approach, ATRP was applied by Müller and coworkers for the synthesis of amphiphilic cylindrical polymer brushes.¹¹⁷ Diblock copolymer side chains were grown from a macroinitiator, PBIEM, yielding well-defined amphiphilic core-shell polymer brushes with *e.g.* PS-*b*-PAA or PAA-*b*-PS side chains. These unimolecular wormlike micelles showed a unique response to solvent quality, as indicated by ¹H NMR and DLS measurements.¹¹⁷ The DLS study verified the unimolecular nature of the Bb molecules in MeOH and MeOH/CHCl₃. In pure MeOH the PS in the core collapsed, while in the presence of CHCl₃ it retained its extended conformation. CONTIN analysis of DLS recorded with multiple angle detection showed an increase in hydrodynamic radii (R_h) from 54.5 ± 1.2 to 60.4 ± 1.3 nm. The observed change was small but significant, indicating that the increase in width is also reflected in the (R_h), which for the large aspect radius of the Bb should be mostly dominated by the length of the wormlike molecule.¹¹⁷

Figure 2.10: Schematic representation of the conformational transitions of PT-*g*-PDMA upon changing the environment from nonpolar organic solvents to water, and following a pH change from 8 to 2 [Reprinted with permission from a reference.¹¹⁵ Copyright (2012), American Chemical Society].

Responsiveness to multiple stimuli can in general be achieved in molecular brushes by attaching different types of polymer side chains¹²³ that respond to different stimuli or by connecting them as copolymer chains to the backbone.¹²⁴ Amphiphilic ethyl cellulose brush polymers with dual side chains,¹²³ poly(2-(2-methoxyethoxy)ethyl methacrylate)-*co*-oligo(ethylene glycol) methacrylate (P(MEO₂MA-*co*-OEGMA)) and PDMAEMA, were synthesized by a combination of ATRP and click chemistry. Each type of side chain, attached to the ethyl cellulose backbone, expressed LCST behavior at a different temperatures. When the temperature was increased to above the first LCST finite-size aggregation occurred, which upon exceeding the second LCST further increased.

Rare examples of a molecular brush possessing a stimuli responsive backbone are described by McCarly¹¹⁴ and by Winnik.¹¹⁵ Both groups prepared a polythiophene

(PT) macroinitiator from which temperature responsive PNIPAM¹¹⁴ or pH responsive poly(*N,N*-dimethylaminoethyl methacrylate) (PDMA)¹¹⁵ chains were grown by ATRP. In case of the temperature responsive PT-*g*-PNIPAM molecular brushes, static light-scattering experiments in water demonstrated that at temperatures above the LCST, the radius of gyration of PT-*g*-PNIPAM was approximately 50% of that observed at temperatures below 32 °C (70 nm vs. 150 nm). Light-scattering measurements also suggested that the shape of the molecules changed from a random coil to a collapsed sphere upon crossing the LCST. The PT-*g*-PDMA molecular brushes exhibited a reversible response to pH changes in water. DLS measurements showed that the extent of protonation of the PDMA side chains influenced the extension and contraction of the backbone, which in turn was reflected by changes in the absorption and fluorescence spectra of the polymer solution. A schematic representation of the proposed conformational transition is displayed in Figure 2.10. At pH = 8, molecules form finite-size aggregates in solution, indicated by the obtained size values of $R_h^{app} = 95$ nm. These aggregates break up at pH $\sim$ 2 and individual molecules are present in the solution, with an apparent hydrodynamic radius $R_h^{app} = 46$ nm.

Figure 2.11: AFM height images of extended and collapsed PBIEM-*g*-PNIPAM bottlebrush, spincoated from aqueous solution (a) below and (b) above the LCST [Reprinted with permission from the corresponding publisher of the reference¹⁰⁴].

The transition of a cylindrical brush polymer to a collapsed structure can usually be visualized by AFM due to the large size of the grafts. As was shown in the above discussed examples, in dilute solution the LCST behavior is expressed by the collapse of individual macromolecules resulting in a compact structure due to the relatively

large distances between the molecules. AFM images¹⁰⁴ of extended and collapsed PBIEM-*g*-PNIPAM bottlebrush molecules are shown in Figure 2.11. The PBIEM-*g*-PNIPAM molecules were spincoated from aqueous solution at 20 °C and 38 °C onto mica surfaces. No significant differences were found between the measured sizes in dry and in wet state, indicating that the conformation of the cylindrical brush polymers did not change during the drying process. The measured average size ($L = 110$ nm) of the molecular brushes obtained from the AFM investigation was somewhat smaller than expected from the light scattering measurement. Careful calculation by compering the well resolved backbone-coron from AFM images in air compared to the images obtained in liquid, resolved the discrepancy. It was suggested that most probably in liquid media the side chains are collapsed or that due to limitations in imaging resolution the measured size is smaller than it should be.

A series of water-soluble, loosely grafted PAA molecular brushes were adsorbed onto mica surfaces and AFM was used by Sheiko *et al.*¹⁰² to study the globule-to-extended conformation transition of the brush molecules as a function of pH. The pH induced conformational changes in relation to the grafting density were investigated. The loosely grafted PMMA-*s*-(PBPEM-*g*-PAA) molecular brushes underwent a globule-to-extended conformation transition in response to increasing pH of the aqueous environment. The conformational behavior was compared with 100%-grafted PAA brushes. Unlike the loose brushes, the 100%-grafted molecules demonstrated a fully extended conformation in a broad range of pH values (pH = 2-9) due to steric repulsion of the densely grafted side chains, which is significantly enhanced upon adsorption onto the substrate. Complementary DLS measurements showed decreasing R_h values upon decreasing the grafting density until a certain limit, while further lowering the grafting density resulted in no further change in the hydrodynamic size.

A fundamental advantage of AFM is that it has the possibility to observe macromolecular motion on a surface in real time. Sheiko and coworkers showed in a series of publications^{121,125-127} the vapour induced conformational transitions of single poly(butanoate-ethylmethacrylate)-*graft*-poly(n-butyl acrylate) p(BEM)-*g*-p(BA) bottlebrush molecules deposited on various surfaces. Reversible coil-to-globule conformation changes of individual molecules (Figure 2.12) were followed upon changing the surface tension by introducing different vapors into the measurement chamber in cyclic exposure. According to these workers, the interaction forces between the macromolecules and the surface changed due to coadsorption of the vapor molecules. They found that the compacted globules were slightly shifted after each cycle. When the molecules were deposited onto SrTiO₃ instead of mica or HOPG, they observed that the nanoscopic surface relief of the substrate affects the macromolecular conformation and promotes orientation of the extending macromolecules along the direction of the surface facets.

Motion of molecular bottlebrush molecules can be induced by, for instance, mechanical tension. Also in the work of Sheiko *et al.* spreading of a drop of polymer

Figure 2.12: AFM micrographs of individual p(BEM)-g-p(BA) brush molecules on SrTiO₃ faceted surface during exposure to vapours of different alcohols. The images show the same molecules at the initial stage of the coil-to-globule transitions of six different collapse-decollapse cycles: (a-1st, b-4th, c-6th, d-9th, e-10th, f-12th collapse). The transitions were induced by humidified ethanol vapour. Scan size: 750-750 nm, bar size: 100 nm, height scale: 3-5 nm. Several different individual macromolecules are highlighted by different colours [Adapted from a reference¹²¹].

bottlebrush melt was studied by in situ AFM scanning.¹²⁸⁻¹³⁰ By capturing images continuously (see Figure 2.13) spreading rate of the precursor film, the flow induced diffusion rate of the molecules as well as the thermal diffusion coefficient and rotational constant of single molecules were independently determined. The movement of the molecules was assigned to be Brownian motion, however among the edges of the underlying HOPG surface, the direction of flow was influenced. By monitoring brush-like macromolecules as they change their shape in response to variations in the film pressure during flow and after appropriate calibration, these molecules can be used as molecular sensors to gauge both the pressure gradient and the friction coefficient at the substrate.¹²⁹ A drawback of the technique though is the sensitivity of the molecules, which may break during spreading due to the high mechanical stress exerted on the backbone.^{131,132} By designing controlled disintegration of the molecules, e.g. via incorporation of weak linkages, this phenomenon can be exploited and the brush-like macromolecules can be utilized as molecular tensile testing machines.¹³³⁻¹³⁵

Figure 2.13: (a) Spreading of a melt of brush-like macromolecules. AFM was used to monitor spreading of a drop of a polymer melt on a solid substrate. The spreading begins with a molecularly thin precursor film that emerges from the drop, which acts as a reservoir for the film. (b) AFM monitors sliding of the precursor monolayer of PBA brushes on the HOPG surface. The images were captured at different spreading times: 10, 80, and 160 min [(a) Reprinted with permission from the corresponding publisher of the reference¹²⁸ (b) adapted from a reference¹²⁹].

2.2.4 Stimulus responsive behavior of polymer chains by AFM-SMFS

For functional materials it is of interest to explore the dynamic behavior of their molecular constituents under varying environmental conditions. Stimulus responsive behavior of the constituents must be understood and eventually controlled. This need has triggered recent interest in AFM based single molecule force spectroscopy (AFM-SMFS) of 'smart' macromolecules. Due to the precise positional control in each direction AFM-SMFS is used for the detection and mechanical characterization of single molecules under environmentally controlled conditions.^{136,137}

In an aqueous solution of PNIPAM, increasing the temperature from below the LCST to above it, a coil-to-globule transformation takes place at the transition point, due to rearrangement of interactions among the polymer chains.¹³⁸ Hence, it is of interest to observe the single chain behavior of PNIPAM 'from a true molecular perspective' via single chain force spectroscopy, specifically as a function of temperature. The first single chain room temperature PNIPAM stretching experiments have been reported on by Zhang *et al.*¹³⁹ (see Figure 2.14). The experiments were done in water and in an 8 M urea solution. The force-extension curves were fitted by the modified freely jointed chain model (m-FJC, for a detailed description of the model see section 2.3). The values of the segment elasticity (K_s) and the Kuhn segment length (l_k) increased when the solvent was changed from deionized water to a 8 M urea solution, from 25 ± 2 N/m to 40 ± 5 N/m and from 0.70 ± 0.05 nm to 0.78 ± 0.06 nm, respectively. In addition, the samples were heated to 33 °C, equili-

Figure 2.14: Force-extension traces obtained of PNIPAM (a) in aqueous solution at room temperature and (b) in 8 M urea solution [Adapted with permission from a reference¹³⁹ Copyright (2012), American Chemical Society].

brated for 10 minutes and subsequently cooled to RT prior to the SMFS experiments. Unfortunately numerical fitting regarding values of chain-statistical parameters was inconclusive. According to the explanation, provided by Zhang *et al.* during the temperature treatment the adsorption of the chains to the substrate changed. This was considered as a severe limiting factor to reproduce the initial force curves (that were obtained prior to the temperature treatment). This issue is indicative of the great importance of chain attachment conformation, segment arrangement, and physisorption to the AFM tip during such 'fishing' type of AFM-SMFS experiments, *i.e.* when no tethering of the chain to the AFM tip is done. The question thus remains open whether the physisorbed chains statistically regained their conformation following the heat treatment. In Chapter 4 we will describe temperature dependent single chain stretching experiments using AFM tip grafted chains.¹⁴⁰ In short, we performed variable temperature single-chain AFM force spectroscopy experiments on PNIPAM to elucidate the role of H-bonding on the stiffness of isolated macromolecules. In these experiments, PNIPAM chains were grafted to Au coated AFM tips using thiol-functionalized chains, and their stretching behavior was monitored. No change in the single chain force-extension curves below and above the LCST was observed. This result indicates that for PNIPAM chains under mechanical stress the formation of intrachain H-bonds at $T > T_{LCST}$ was suppressed.

By variation of the pH of the media, PAA and PMAA responses can be triggered. As a response to the pH change these polymers become negatively charged by deprotonation at $\text{pH} > \text{pKa}$. A notoriously difficult analytical question in characterizing polymer brushes is related to the determination of the chain length values in the brush.⁷¹ Recently Ryan *et al.*¹⁴¹ reported on PMAA brushes and chain length of

macromolecules obtained using a RAFT polymerization and 'grafting from' approach were characterized by AFM-'SMFS' (The quotation mark here refers to the still open question whether the corresponding AFM chain stretching experiments were indeed performed on single chains.). The measured single-molecule force distance curves were fitted using the worm like chain (WLC) model. The values of the contour length (L_c) of the polymer were easily obtained from the fitting as the authors used a fixed persistence length (l_p) value (of 0.5 nm, in deionized water) adapted from the literature, which was measured by small-angle X-ray scattering. In principle, the value of l_p can be obtained from force curve fits. However, due to (i) experimental noise, (ii) uncertainties of the 'zero' point of the stretching curve, (iii) the level of zero force, and (iv) uncertainties of the value of the extended chain length of the given macromolecule stretched, the numerical values derived by fitting all relevant parameters in the various single chain models in AFM-SMFS force-extension curves may be plagued by significant error. By assuming a physically substantiated value for l_p the authors circumvented in part these difficulties. In contrast, in studies by Hadziioannou *et al.*,¹⁴² using individually grafted chains and performing experiments under the same conditions as Ryan *et al.*¹⁴¹ obtained a value of the persistence length by fitting of 0.28 ± 0.05 nm using the WLC model. We note that both groups reported an accurate fitting.

Hadziioannou *et al.* used thiol terminated PMAA chains grafted onto a gold substrate surface¹⁴² with a low grafting density surface in order to increase the chance of single chain stretching events (Figure 2.15). The experimental data were fitted by both, the FJC and the WLC models. The l_k values derived had a magnitude of 0.33 ± 0.05 nm, which is in the same range as the values of l_p which reported to be to 0.28 ± 0.05 nm. According to the FJC and WLC models, this virtual agreement raises a fundamental question. Under the experimental conditions used, and assuming that the models are valid, the value of l_p should be half of that of the l_k .¹⁴³ We note that, nevertheless, the L_c value obtained in this study is in a good agreement with the chain length derived from gel permeation chromatography experiment. Regarding the chain chemical structure, PAA differs from PMAA only by one methyl group substituent. Due to the similarity in the chemical structures one would expect similar stretching properties. In a study reported by Li *et al.*¹⁴⁴ PAA force-distance curves (Figure 2.15) were fitted using the FJC and the m-FJC models. It was shown that the macromolecular stretching is not just entropy controlled, as the use of the simple FJC model did not provide an accurate fitting. The resulting value for the l_k was equal to 0.64 ± 0.05 nm for both FJC and m-FJC.

As mentioned earlier, the properties of the solvent (hydrophilicity, ionic strength, pH) have a great influence on the l_p and the l_k values as *e.g.* summarized in reference.¹³⁷ This may account for the observed different behavior of the two polymers as PAA stretching experiments were performed in 10^{-3} M KNO_3 solution, whereas the PMAA testing was conducted in deionized water. Another noteworthy point is related to the fitting quality. Like mentioned, while for PMAA the simple FJC model described the stretching well, the fitting quality was poor for PAA. This dif-

Figure 2.15: Force-distance curves of a) PAA and (b) PMAA and their fit accordingly to m-FJC and WLC models, respectively [Adapted with permission from references. ^{142,144} Copyright 2012, American Chemical Society].

ference was assigned to enthalpic contributions which must be considered for higher stretching forces.¹⁴⁴ The choice of a threshold force value above which enthalpic contributions must be considered remains somewhat arbitrary, depending also on the primary structure of the macromolecule. However, as a reasonable approximation, one can assume that tensions at forces below ca. 20 pN do not cause torsions in bonding angles that would necessitate the use of enthalpic terms.¹⁴⁵ We note, that for an elastic rod model, Odijk puts the threshold value at 10 pN,¹⁴⁶ although he mentions that the regime that demarcates the entropy from enthalpy dominance is not sharply defined (no pure entropic or enthalpic domains exist).

Reversible conformational changes and stimulus responsive behavior can also be triggered by light.^{147,148} Azobenzenes are well-known chromophores, which can exhibit either trans or cis conformations that can be reversibly switched with light of the appropriate wavelength. The energy barrier separating the two isomers has a height of $40 k_b T$ with the trans state having a lower free enthalpy. Cis-trans isomerization can be achieved by optical excitation at a wavelength of 420 nm via the first excited singlet state. A trans-cis transition via the same excited state can in turn be triggered at a somewhat higher energy corresponding to a 365 nm wavelength radiation. AFM-SMFS experiments demonstrated that chains with cis isomers in their backbone remain stable for all experimentally accessible pulling forces, within the experimentally accessible timescale (the timescale is relevant due to the loading rate dependence of transition forces).¹⁴⁹ Gaub *et al.* exploited the bistability and reversible transitions of the polyazopeptide for constructing a 'macromolecular motor' which converted optical energy into mechanical work in 'opto-mechanical' cycles (Figure 2.16).

Initially, single chain behavior of the polyazopeptide was fitted by the modified Marko-Siggia WLC model. The fitting parameters included l_p , K_o and L_c . The values

Figure 2.16: The traces to the right portray the force – extension of a single polyazopeptide. By the application of five 420-nm pulses, the polymer was switched to the saturated trans state and lengthened, after five pulses at 365 nm, the same molecule was shortened. Traces to the left correspond to a single polyazopeptide strand being shortened against an external force. Inset: schematic of the experimental setup. TIR: total internal reflection. [From a reference.¹⁴⁷ Reprinted with permission from AAAS].

of l_p and K_o were first determined with 'reasonable' WLC fits. The contour lengths of the chains stretched in a given experiment were calculated using these values. The authors assumed that the cis-trans isomerization did not change the persistence length and the segment elasticity. In a subsequent study¹⁵⁰ they extended their analysis to include a freely rotating chain model combined with quantum mechanical calculations. In the azobenzene peptide chain there are three structural units, corresponding to three building blocks *i.e.* the cis-azobenzene, the trans-azobenzene, and the tri-peptide. Under tension the effective bond lengths vary, and this variation for the three types of bonds were calculated by *ab-initio* methods as a function of force (QM-modification).¹⁵⁰ Using this method the number of cis and trans units along the chain could be estimated and it was successfully employed to assess the opto-mechanical conversion efficiency of isomerization processes when the chain was irradiated to induce cis-trans (or trans-cis) isomerization. Results obtained also explained why the thermal relaxation of the cis conformer did not speed up under tension, thus explaining why forces up to 500 pN did not change the partition of the cis and trans conformations during experiments of several seconds.

The last group of stimulus responsive polymers that are discussed here is the redox responsive PFSs. Zhang *et al.*¹⁵¹ studied the elastic properties of two different

PFS derivatives, dimethyl (PFS) and methyl - phenylsilane (PFMPS), as well as their chemically oxidized (with FeCl_3) forms. SMFS force-extension curves were measured in a droplet of tetrahydrofuran (THF). Fitting by the m-FJC model resulted in different values of l_k and K_s , depending on the degree of oxidation and substitution. By changing one of the methyl groups to a phenyl group, the l_k fitted value decreased (0.41 ± 0.01 nm to 0.36 ± 0.01 nm) but the K_s value increased from 53 ± 1 nN/m (PFS) to 61 ± 1 nN/m (PFMPS). Oxidizing the polymer made a more significant change in the K_s (115 ± 1 nN/m for PFS, 500 ± 1 nN/m for PFMPS), while the increase in the value of the l_k was relatively less significant (0.46 ± 0.01 nm for PFS, 0.40 ± 0.01 nm for PFMPS).

Figure 2.17: Realization of a molecular motor based on PFS_{100} , measured by electrochemical AFM-SMFS. The mechanical work (output) of the cycle corresponds to the effectively converted electrochemical potential. Starting from a force of 20 pN (point 1) under a potential 0.5 V, an individual, oxidized PFS polymer chain of 50 nm contour length is pulled to a force of 140 pN (point 2). At a constant force of 140 pN, the PFS chain is reduced to its neutral state by setting the potential to 0 V (point 3), to result in a change of the elasticity of the polymer chain. The force on the polymer is then reduced back to 20 pN (point 4). Finally the cycle is completed by changing the potential to 0.5 V in order to completely oxidize the PFS chain. [Adapted from references^{152,153}].

Vancso *et al.* described AFM-SMFS on PFS in a subsequent study under electrochemical control.^{76,152} In their experiments ethylene sulfide end-capped PFS_{100} (the subscript refers to the number average degree of polymerization) was covalently attached by grafting onto a gold surface, by inserting the chain into the defects of a self assembled, inert monolayer. This allowed studies of separated, single chains. In order to compare differences of elasticity between neutral and oxidized

PFS macromolecules under electrochemical control, individual chains were probed by AFM-SMFS. Since the targeted electrochemistry experiments were carried out in aqueous solutions, the single chain stretching of neutral PFS was also performed in aqueous solutions of NaClO_4 for comparison. From the fits of the force-extension curves it was concluded that the m-FJC model describes both entropic (both low and high force regime) and enthalpic (high force regime) elasticities of all types of PFS polymer chains very well. In this work, an increase in l_k (from 0.40 nm in the neutral state to 0.65 nm in the oxidized state) of the PFS was observed, which was attributed to electrostatic repulsion. This contribution originates from the positive charges that are distributed along the polymer chains. According to the classical Odijk-Skolnick-Fixman theory,^{154,155} it is expected that the electrostatic interactions between the charges along the polymer chain increase the distances between like-charged segments, *i.e.* the stretched conformation of the chains is favored. The resulting increase in Kuhn segment length corresponds to a lower restoring force or elasticity of the oxidized PFS. We note that incomplete oxidation by chemical oxidants caused a smaller variation of the single chain statistical parameters, as compared to full electrochemical oxidation.

Zhang *et al.*,¹⁵¹ who used FeCl_3 as chemical oxidation agent for PFS homopolymers, reported a substantial increase in the enthalpic elasticity for PFS. The Kuhn segment length increased by 10% following oxidation, and the value of the segment elasticity was more than doubled. These data indicate, in agreement with the data of Vancso's group, that the elasticity of individual PFS macromolecules was changed after oxidation.

In addition to fundamental scientific interest in single chain behavior, there are also potentially relevant technological consequences of AFM-SMFS on macromolecules. For example, for future functional nanoscale devices, such as pumps, valves, levers and molecular walkers, molecular-scale motors will be required to power these structures. Moreover, a complete understanding of the working principles of biological molecular motors *e.g.* of motor proteins and their use in artificial muscles, need further research. These issues have motivated work aiming at single chain molecular motors as shown in [Figure 2.16](#) and [Figure 2.17](#).

2.2.5 Surface interactions studied by AFM

Mapping of chemical contrast at heterogeneous polymer surfaces based on adhesive forces

There is compelling evidence, that AFM has become a true enabling platform in polymer science and specifically in macromolecular nanotechnology. AFM has gone well beyond 'just' being a technique for imaging.¹⁵⁶ The basic utility of contact AFM is to measure force-distance curves (besides obtaining topological information). It is well known from contact mechanics¹⁵⁷ that the adhesion force F_{adh} between a spherical particle (apex of the tip) and a flat surface is controlled by the interfacial

tension of the contacting materials. As the interfacial tension is a material specific constant, in principle by measuring adhesion forces one could imagine the identification of materials in contact from surface forces using a spectroscopic approach. Attempts to use force spectroscopy for polymer identification have been made and are reviewed in the literature.¹⁵⁸ However, as it was pointed out early on by Spencer *et al.*¹⁵⁹ a quantitative correlation between measured pull-off force (force of adhesion) and theoretical work of adhesion exist for non-polar tip-sample combinations only. When polar polymers are considered, not even the order of magnitude of interaction strengths could be predicted. The situation is further complicated when specific interactions should be taken into account. Even for dispersive interactions, the interaction strength had to be optimized by using perfluorodecaline as imaging medium to maximize measured force contrast. One may conclude that quantitative force spectroscopy for polymer identification remains elusive. However, for heterogeneous systems, the contact force contrast allows one to laterally map surface compositions and thus visualize phase distribution of unknown specimens.

During contact mode imaging surface tribological properties can be obtained. When the scanning direction is perpendicular to the long axis of the cantilever static and dynamic friction can be captured as the measured surface tangential forces are related to tribological properties.¹⁵⁸ When chemically modified AFM probe is used the differences are enhanced. To determine the distribution of surface chemical groups lateral mapping, in the so called force volume imaging mode, can be performed with near nanometer (several tens of nm) resolution when sharp probes and appropriate imaging conditions are used. Force volume imaging is based on adhesion force measurement by chemical force microscopy, where the collected data is displayed in an image, as shown in Figure 2.18.

The major drawback of force volume imaging is that it is time consuming and due to the development of new methods (harmonix and peak force tapping (see in section 2.5.2)), where the adhesion map of the surface is recorded during scanning, the use of the 'basic' force volume imaging is suppressed. Since force-volume imaging related studies substantially and well reviewed,^{158,160,161} here we show only one recent example.

The surface chemistry and ionization state of cross-linked poly(dimethylsiloxane) (PDMS) exposed to UV/ozone were studied as a function of treatment time (see Figure 2.18).¹⁶² The use of a chemically modified tip surface enhanced the differences between the surface exposed moieties. The interactions between the ionizable functional (silanol) groups - formed during the treatment - and the hydroxyl groups - at the tip - influenced the magnitude and the pH dependence of the recorded pull-off forces, in solutions with various ionic strength. Figure 2.18 shows a clear difference of the surface properties after the treatment, *i.e.* the high adhesion forces at pH = 3 practically vanished at pH = 7.2. Force titration curves, monitoring the tip-sample adherence as a function of pH, allowed the authors to construct a map of the pK_a values of the functional surface groups with a ca. 20 nm spatial resolution.

As mentioned above, high resolution composition image with harmonix or peak

Figure 2.18: Contact mode AFM force volume images of a PDMS surface after 30 min of UV/ozone treatment in phosphate buffer solution (ionic strength = 300 mM) at (a) pH = 3, $F_{pull-off} = 8.06 \pm 0.97$ nN and (b) pH = 7.2, $\pm = 0.20 \pm 0.10$ nN. The color scale ranges from dark (high adherence) to bright (low adherence). Scan size: 500 nm x 500 nm. The Z range of the force volume was 20 nm. (c) Typical force curve from a region with (1) low and (2) high adherence at pH = 3. (d) Typical force curve obtained for the same surface at pH = 7.2. [Reprinted with permission from a reference.¹⁶² Copyright (2012), American Chemical Society].

force tapping is easily obtained, however the interest in these methods lies in their substantially enhanced accuracy and precision in quantitative determination of surface elastic modulus values. As the value of mechanical modulus is also composition dependent, component identification could in principle be performed (with limitations) using quantitative data of Young's moduli. Variation of the range of numerical moduli however primarily depend on the physical state, morphology and other structural parameters, thus posing clear limitations.^{163,164} Description of mechanical models is provided in section 2.3. These dynamic AFM imaging modes, *i.e.* harmonix and peak force tapping, are appropriate to assess the mechanical performance

of phase separated polyurethanes^{165,166} quantitatively as a function of their molecular structure (Figure 2.19). The values of surface elastic moduli were determined with nanoscale resolution and were in excellent agreement for both of these AFM modes. While other commonly used measurements such as tensile tests provide a bulk average value for the elastic modulus, AFM based elastic moduli mappings enable the study of surface stiffness with nanoscale resolution in a quantitative way. Another comparative analysis¹⁶⁷ between these methods to obtain surface properties

Figure 2.19: Mechanical modulus maps (according to the DMT theory) as obtained by harmonix (A, C, E) and peak force tapping (B, D, F) of three different polyurethane composite sample (A, B: Sample 1, C, D: Sample 2, E, F: Sample3), of different compositions; The color code corresponds to variations in the values of Young's moduli; similar morphology of the same samples were observed with the different techniques; Scan sizes: $2 \times 2 \mu\text{m}$. z-scales: (A, B), 600 MPa; (C-F), 1 GPa [Reprinted from a reference¹⁶⁵, Copyright (2012), with permission from Elsevier].

showed essentially no difference for the measured values. For α -synuclein amyloid fibrils modulus values studied with conventional phase imaging (see tapping mode in section 2.4), harmonix and peak force tapping imaging were in each others error range.

Limitations of the harmonix method include the quantitative mapping of phases with a large difference in surface moduli (10 MPa -10 GPa). The peak force method covers a wider range of modulus values that can be measured, from hard surfaces

($E=70$ GPa) to soft biological samples ($E=0.7$ MPa) even in liquid environments. In spite of the advantages of peak force over harmonix one has to be careful choosing a tip that is appropriate to the sample under study.¹⁶⁸

The geometry of the AFM tip plays an important role in the data analysis. It is generally approximated as spherical at the very end. Adhesion often comprises a portion of the tip-sample interaction force. This configuration fits well with the DMT (Derjaguin-Muller-Toporov) model being suitable for samples with moderate adhesion levels and sharp tips. The JKR (Johnson-Kendall-Roberts) model is more detailed and also frequently used to derive local elastic moduli, is more appropriate for very soft, very adhesive samples or when AFM tips with larger radii are used, and as such it provides better results on compliant samples (1-10 MPa). Importantly, the JKR model requests a more complicated computation, rendering it less favorable for real time imaging. Offline calculation and comparison of the same data using the DMT and JKR models showed about 15% deviation.¹⁶⁹

The AFM tip geometry, its penetration depth and hence the contact area have a pronounced influence on the measurement of the sample moduli. A part of it is taken into account in the calibration procedure with a microphase separated polymer with known moduli.¹⁷⁰ The same setpoints should be chosen for the calibration and for the sample measurements. In addition, local topography or roughness can have impact on the measured moduli due to variation in contact area.

Colloid probes in AFM measurements

Morphology and chemical-physical properties of large surfaces are nowadays characterized using various AFM based methods such as harmonix or peak force scanning, as discussed in detail in the previous section. Still these enhanced methods are not appropriate for the characterization of the surface properties of small, few micron sized particles. Investigation of particle (-particle) interactions at the nanoscale became accessible when the first colloid probe measurement was introduced by Ducker.¹⁷¹ Since then, it is a widely used technique to probe interactions between surfaces, thus making single particle experiments feasible.¹⁶³

Both, in air and in aqueous environments, the colloid probe method is appropriate to establish physico-chemical properties of colloid particles across the medium. This information is beneficial in numerous applications, e.g dispersibility of colloid particles in liquid media, for printing applications, paint stabilization.¹⁷² From another point of view this technique allows one to measure surface forces quantitatively since the radius of the microsphere can be determined with a better precision than the radius of a conventional AFM tip. The most commonly used colloid probes are made of SiO_2 particles, but recently polymer colloid particles become more commonly used as well. Furthermore the technique enables one to study interactions upon polymer-polymer contacts, since the relatively large surface area of the colloid tip serves as a platform for easy chemical modification. Polymers,¹⁷³ proteins,^{174,175} etc. can be physisorped or chemically attached on the various colloid particles like SiO_2 ,^{173,174}

Au^{176,177} or polymer matrix.^{178–180}

Evaluation of the interaction force acting upon approaching the colloid particle to the surface is based on the Derjaguin, Landau-Verwey-Overbeek theory (DLVO).¹⁸¹ The model calculates the contribution of electrostatic (electric double layer) as well as van der Waals forces with respect to the surface roughness of the contacting bodies in liquid environment.¹⁸² The DLVO theory was successfully applied to describe long range interaction between a colloidal AFM probe and the investigated polymer film in solutions of varying ionic strength and pH.^{183,184}

The use of colloid particles in chemical force spectroscopy is rapidly developing as well, since information about the surface adherence properties is obtained over a large area. The developed adhesive forces between the colloid particle, attached to the cantilever, and the polymer surface can be measured via pull off forces^{161,185} upon separation (Figure 2.25, position 3-5). Adhesion force measurements, carried out with SiO₂ particles, are often used for characterization of polymer coatings,^{186–188} anti-biofouling surfaces,^{189,190} describe electrostatic properties of charged polymer coatings¹⁸³ or to determine aggregation forces between drug carrier colloid particles.¹⁹¹ Under various environmental conditions, the obtained adhesion forces can give information about interfacial forces of polymer layers associated with the different surfaces,^{192–194} as well as about phase transition behavior, which is accompanied by hydrophilic-hydrophobic changes, of temperature responsive polymer surfaces as it was studied for PNIPAM^{195–197} and PEG.¹⁹⁸

Utilizing polymer spheres as colloid probes allows one to study surface properties of the polymer surfaces of interest in a direct manner, avoiding modification steps of the probes.¹⁷² Polymer colloid probes are commercially available, however there are easily accessible microfluidic applications to create low polydispersity polymer particles¹⁹⁹ of the polymer of interest as well. The as such obtained spheres still have to be glued to the cantilever. Recently, interaction forces between components of composite materials such as xyloglucan - cellulose,^{200,201} or poly(lactic-co-glycolic acid) (PLGA) - cellulose were probed,²⁰² as the required cellulose spheres became available by the so called viscose process.

To probe rheological properties it is 'essential' in some cases to employ colloid spheres instead of the conventional AFM tip. Since typical values of AFM tip radii are in the range of several tens of nanometers, while probing rheological properties of soft polymers, especially polymer brush samples, one may experience that tips penetrate into the brush due to high local pressure.^{203,204} This is circumvented by the use of colloid particles with radii on the order of micrometers.²⁰⁵ Thus colloidal probe AFMs benefit from the knowledge of the geometry of the probe and distributes the pressure on the sample surface over a larger contact area, while the force sensitivity of the AFM is maintained. We note however, that if a high resolution mapping of the elasticity properties is required, *e.g.*: force volume imaging with conventional AFM tip can be used.²⁰⁶ The mechanical response of macromolecular structures, with dimensions in the range from a few nanometers to several micrometers, to external forces plays a fundamental role in many biological and cellular processes, as well as

in synthetic polymers. Thus AFM colloid probe measurements are widely used to characterize biological systems as well, for instance to determine stiffness of blood-cells²⁰⁷ and cells.^{208,209} Assessment of the forces required to stretch or compress macromolecular structures is of importance for mechanical devices.

To gain insight into the physical phenomena that govern the mechanical behavior of polymer brushes, recently several colloid probe compression studies have been reported. Surface and elastic properties as a function of structural parameters such as grafting density were measured for temperature responsive PNIPAM brushes,¹⁹⁵ showing an increased apparent stiffness of the polymer brush upon decreasing the grafting density. Similarly, deformation curves obtained from PEGMA brushes revealed a clear trend of the elastic behavior upon varying the grafting density.²¹⁰ Compression of negatively charged low density polymer grafts composed of hyaluronan over a wide range of ionic strengths was studied.^{211,212} Analysis of the recorded force-distance curves provided valuable information of statistical physical parameters, the intrinsic chain stiffness and the statistical segment length were determined, as well as the influence of inter- and intra-molecular forces on the behavior of the polymer grafts.

The colloid probe technique was developed originally for hard particles, enabling one to gain information about polymer 'droplet' deformation due to its high vertical sensitivity. It is essential for the measurement to ensure that the size of the probe particle is larger than the investigated system²¹³ to avoid penetration and uneven pressure distribution. As an example, a study of the deformation of cross linked PDMS droplets by a SiO₂ probe was carried out and the result pointed out that the nano-rheological properties of the system were controlled by the bulk rheology rather than the droplet's interfacial properties.^{214,215} Viscoelastic deformation of colloidal spheres is based on several theoretical models of contacting surfaces, but one must be careful when choosing the model, as neglecting electrical double layer forces may introduce a significant error. Elastic properties, such as the compression modulus, can be determined from the approach curve based on the Hertzian model, or in more complex cases the JKR or DMT models can be applied.

Extended use of soft particles in colloid probe measurements provide a tool to characterize elastic behavior of the particle attached to the cantilever,²¹⁷ as well as to investigate the developing contact area between the contacting surfaces. Fery *et al.*^{209,216,218,219} reported on the use of a setup where AFM and reflection interference contrast microscopy (RICM) were combined, thus the deformation of the microsphere is directly monitored simultaneously with the applied force (Figure 2.20). The elastic parameters and the thermodynamic work of adhesion of the colloid particle were calculated with the JKR approach, since the contact area was measured as a function of the applied load. In addition the method is appropriate to characterize contributions of capillary forces in air, and hydration forces and hydrophobic interactions in water. It is important to note that the main difficulty with AFM measurements on deformable systems is the accurate determination of the zero distance. Usually there is no sharp transition between the contact and non-contact regions,

Figure 2.20: (a) Schematic of the experimental setup reported by Fery *et al.*: combination of AFM and optics. Analysis of the thermodynamic work of adhesion by extraction of the contact radius for varying loads. (b) Force-distance curve of a hydrophilic soft colloidal probe against a hydrophilic surface (solid line represents approach cycle, dotted line represents the retraction cycle, and dashed lines show readings of the contact area as shown in c). (c) RICM images of the SCP allowed determination of the contact radius. (d) The plot of the contact radius against the load, follows the JKR predictions. The thermodynamic work is extracted from the JKR fits (cross-makers (x) denote experimental data, black line denotes the JKR fit) [Adapted from a reference²¹⁶].

because deformation already occurs prior to contact due to the extended range of surface forces.²¹⁵

2.3 Theoretical models of contact mechanics

Nanomechanical and adhesive properties of surfaces, such as quantification of the elastic modulus, adhesion, inter-/intramolecular forces or elasticity, easily can be propped by atomic force microscopy based methods at the nanoscale.^{105,164} As a surface-based technique, AFM based surface force spectroscopy (SFS), is well suited to study the effect of free surfaces and confined surfaces on polymeric properties, which can be quite different from bulk properties.^{160,161,163} These information can be obtained from the so called force-distance curves, which is a plot of the AFM tip-sample force vs the piezoelement movement. For a detailed description see section 2.5 about AFM and AFM spectroscopies. The deflection of the cantilever, when in contact with the sample surface, mechanical properties such as elastic modulus and hardness can be obtained from the corrected slope of the force curve after contact, while when the contacting surfaces are separated from each other the adhesion force, if there is any, is measured.

When the AFM tip/probe reaches the surface, it is pressed into the surface with a

known force and the deflection of the cantilever is monitored, and recorded on a force-distance curve. In this region, as the piezo scanner moves the sample surface closer toward the cantilever, the cantilever bends upwards. For infinitely stiff substrates the slope is 1, which reflects the fact that the cantilever deflection is exactly equal to the piezoelement displacement. This region is linear for purely elastic deformation with a slope directly related to surface stiffness. The indentation depth (the sample is in contact with the tip) is the difference between the separation distance that would be obtained on a hard surface compared to the separation distance obtained for a soft sample. The deformation (δ) of the surface can be obtained from the approach part of the deflection - distance curves, according to:

$$\delta = |Z - Z_0| - (D - D_0) \quad (2.2)$$

where Z denotes the piezo-extension, D is the value of deflection of the cantilever, Z_0 and D_0 are values of an offset at which the tip starts to contact the sample surface. The piezo-extension and the cantilever deflections are related to the stiffness of the sample and the cantilever the following way:

$$k_s \delta = k_c (Z - Z_0) \quad (2.3)$$

where k_s and k_c are the stiffness of the sample and the cantilever, respectively. From equation 2.2 and 2.3 the relation of the sample stiffness to the applied force (F), based on the Hooke's law, can be determined.

$$F = k (D - D_0) = -\frac{k_c k_s}{k_c + k_s} (Z - Z_0) = k_{eff} (Z - Z_0) \quad (2.4)$$

where, k_{eff} is the combined stiffness of the sample and cantilever. If the cantilever is much stiffer than the sample $k_c \gg k_s$ then $k_{eff} \approx k_s$. Furthermore, the stiffness of the sample is related to its Young's modulus by

$$k_s = \frac{3}{2} a E_{tot} \text{ and } \frac{1}{E_{tot}} = \frac{3}{4} \left(\frac{1 - \nu_s^2}{E_s} + \frac{1 - \nu_t^2}{E_t} \right) \quad (2.5)$$

where, ν_t , E_t , ν_s and E_s are the Poisson's ratio and the Young's moduli of tip and sample, respectively, E_{tot} is the reduced Young's modulus and a is the tip-sample contact radius. If the tip is much stiffer than the sample, its deformation can be neglected, thus the sample stiffness can be approximated by

$$k_s = 2a \left(\frac{E_s}{1 - \nu_s^2} \right) \quad (2.6)$$

To calculate the elastic modulus from applied loading force and piezo extension data involves applying a model to account for the tip-surface contact area ($2\pi a^2$). The most common models are the Hertzian model, Derjaguin-Muller-Toporov (DMT) and Johnson-Kendall-Roberts (JKR). For deformable surfaces the

presence of surface forces (repulsive, adhesive) can lead to a significant deformation even before contact is established, that is certainly the case for very soft materials. Thus one must choose the proper mechanical relationships with which to evaluate the data in order to determine the materials properties of the sample as well as the tip-sample contact are. In Table 2.1¹⁶³ the relation between the contact radius a , the sample deformation δ and the adhesion force F_{adh} for a spherical tip on a flat surface according to the above mentioned models.

Table 2.1: Relation between the contact radius a , the sample deformation δ and the adhesion force F_{adh} for a spherical tip on a flat surface according to the different models.

	a	δ	F_{adh}
Hertz	$\sqrt[3]{\frac{RF}{E_{tot}}}$	$\frac{a^2}{R} = \left(\frac{F^2}{RE_{tot}^2} \right)$	0
DMT	$\sqrt[3]{\frac{R}{E_{tot}} (F + 2\pi RW)}$	$\frac{a^2}{R} = \frac{(F + 2\pi RW)^{2/3}}{\sqrt[3]{RE_{tot}^2}}$	$2\pi RW$
JKR	$\sqrt[3]{\frac{R}{E_{tot}} \left(F + 3\pi RW + \sqrt{6\pi RWF + (3\pi RW)^2} \right)}$	$\frac{a^2}{R} = \frac{2}{3} \sqrt{\frac{6\pi W a}{RE_{tot}}}$	$\frac{3}{2} \pi RW$

R is the tip radius, W is the adhesion work per unit area, F is the applied force, E_{tot} is the reduced Young's modulus.

2.4 Theoretical models of polymer elasticity

AFM-SMFS provides information about forces and strains developed during conformational changes in the macromolecule induced by an external force,^{153,220} therefore the force-extension curve recorded during the stretching experiment describes the elasticity of the macromolecule.

Depending on whether the enthalpic or the entropic restoring forces dominate, the stretching path of a single polymer chain consists of different parts. In the initial state, due to Brownian molecular motion, a permanent fluctuation is present, while the chain adopts a random coil configuration. At low applied forces, the number of possible conformations is reduced, the molecule is being extended and loss of entropy occurs. Further uncoiling of the chain results in a stress in the molecular backbone. As the chain exceeds its contour length, corresponding enthalpic elasticity will play in role in addition to the entropic forces.

There are several statistical models that can be applied to obtain quantitative data regarding the behavior of a single polymer chain upon pulling.¹⁴⁵ The confor-

mation of polymer chains in solution fluctuates between different rotational states which possess energy levels that depend on short-range and long-range interactions between polymer segments. The various conformations and associated energy levels are populated according to the laws of Boltzmann statistics.¹⁴³ Due to the relative ease of rotation around bonds, flexible polymer molecules can adopt a large number of possible conformations in solution, leading to a random coil structure. The simplest way to describe a polymer chain is to utilize the freely-jointed chain (FJC)²²¹ (Brownian chain) model. In this model, the chains are composed of an N number of rigid, orientation independent segments (Kuhn's segment) with lengths of l_k , connected via flexible joints, giving the following relationship for the contour length (L_c) of the polymer:

$$L_c = Nl_k \quad (2.7)$$

A more complex model is the worm like chain model (WLC) (Figure 2.21), that takes a certain excluded volume into account, unlike the FJC model. The WLC model describes a polymer chain that is intermediate between a rigid-rod and a flexible coil, and takes into account both local stiffness (described by the persistence length, l_p) and long-range flexibility. The chain is treated as an isotropic, homogeneous rod whose trajectory varies continuously and smoothly through location, thus preventing the units to occupy each possible space with the same probability. If a chain is shorter than l_p , the polymer is considered to be linear.

Figure 2.21: Schematic representation of the FJC and the WLC models. In the FJC model, a polymer chain is composed of N rigid segments (with Kuhn length l_k), while in the WLC model, the entire polymer is represented by an irregularly curved filament, which is linear on the scale of the persistence length l_p .

The pulling force to uncoil the polymer chain in the WLC model^{222,223} is given as a function of the extension, thus it can be applied directly when a force-extension curve is obtained using AFM-SMFS; see equation 2.8:

$$F(z) = \frac{k_b T}{l_p} \left[\frac{1}{4} \left(1 - \frac{z}{L_c} \right)^{-2} + \frac{z}{L_c} - \frac{1}{4} \right] \quad (2.8)$$

where $F(z)$ is the applied force, z is the extension, l_p is the persistence length and L_c is the contour length of the polymer, k_b is Boltzmann's constant and T is the temperature.

Although this approach takes into account both entropic and enthalpic contributions, the extension is limited by the contour length of the polymer (L_c), and the model fails describing macromolecules under conditions of high stress. Extended WLC models have been developed where segment elasticity is taken into account to broaden the range of applications.¹⁴⁶

In case of the FJC model the extension²²⁴ is expressed by the force acting between the ends of the polymer chain as seen in the following equation:

$$X(F) = L \left[\coth \left(\frac{Fl_k}{k_b T} \right) - \frac{k_b T}{Fl_k} \right] \quad (2.9)$$

where F is the applied force, $X(F)$ is the extension, l_k is the Kuhn's segment length, k_b is the Boltzmann constant and T is the temperature. Since the FJC model doesn't consider entropic effects it fails to describe the stretching of molecules for $X \sim L$. In order to optimize the FJC model, a modified FJC model was developed where an additional parameter, the segment elasticity (K_s), was introduced. This additional enthalpic term compensates for the elastic behavior of the chain at large extensions. In the extended model besides K_s , the contour length L_c and the number of segments n are considered as well, expressed in the following way:

$$X(F) = L_c \left[\coth \left(\frac{Fl_k}{k_b T} \right) - \frac{k_b T}{Fl_k} \right] \left[1 + \frac{F}{K_s l_k} \right] \quad (2.10)$$

For the FJC based models fit parameters can only be obtained numerically. For neutral polymers and biopolymers *e.g.* DNA^{221,225} and proteins²²⁶ both the FJC model and the WIC model, and their extensions, describes the stretching behavior very well while WLC can be applied for polymers with a charged backbone. For small extensions, the difference between the WLC and the FJC model is negligible, giving that at low forces, the persistence and Kuhn length are related as follows: $l_k = 2l_p$.

2.5 Characterization methods

In order to characterize/visualize bottlebrush polymeric structures specific tools are needed. To obtain information about the size of polymer chains in solution, as was shown in the previous sections for characterization of the bottlebrush molecules, one can utilize dynamic light scattering measurement. Surface properties of polymer brush surfaces, as well as rheological characteristics at the nanoscale can be characterized by AFM and AFM based techniques.

2.5.1 Dynamic light scattering

In dilute solutions the polymer chains occupy a certain volume.^{32,34,227} The dimensions of a polymer chain can be described by two quantities.³² One option is to use the average root-mean-square end-to-end-distance ($\langle r^2 \rangle$ or $\mathbf{R}$) defined by the end-to-end vector ($\vec{r}$) between the first and last unit of the polymer chain (sketched on Figure 2.21). The other way to describe the dimension of the polymer chain is the root-mean-square radius of gyration ($\langle R_g^2 \rangle$ or R_g), which is the average distance of a chain element from the center of the gravity of the coil. In case of the ideal chain these measures are related in the following way: $\mathbf{R} = 6R_g$.

In a light scattering experiment, a sample, consisting of a dilute solution of molecules, is illuminated by a monochromatic laser light that is scattered into a detector. The illuminated volume is defined by the interaction of the incident beam and the scattered beam. The molecules in the illuminated region are continuously translating, rotating and vibrating due to thermal interaction with the solvent molecules. As a consequence of this Brownian motion the total scattered light at the detector fluctuates in time, giving important structural and dynamical information about the position and orientation of the molecules.^{228–230}

Figure 2.22: Scheme of the DLS setup

The relation between the particle size and the wavelength of laser used in the experiment determines the nature of the scattered light. Either Rayleigh scattering or Mie scattering is applicable. If the particles are small compared to the wavelength of the laser used (around $d = \lambda/10$), then the scattering from a particle is isotropic, (*i.e.* equal in all directions), and described by the Rayleigh approximation. When the size of the particles becomes roughly equivalent to the wavelength of the illuminating light the directionality of the scattered light is anisotropic and forward scattering is more preferred.

The correlation function

The time correlation function, measured in DLS is an ensemble-averaged time-correlation function.²²⁹ The autocorrelation function ($g^{(2)}(\tau)$) of the scattered light intensity ($I(t)$) for a given delay time (τ) is expressed in the following form:

$$g^{(2)}(\tau) = \frac{\langle I(t) I(t + \tau) \rangle}{\langle I(t) \rangle^2} \quad (2.11)$$

where, $I(t)$ and $I(t + \tau)$ are the intensity of the scattered light at time t and $t + \tau$, respectively, the brackets indicate the averaging over t . The correlation is converted to field-field correlation by the Siegert relation,²³¹ therefore:

$$g^{(2)}(\tau) = B + \beta \left[g^{(1)}(\tau) \right]^2 \quad (2.12)$$

where, $g^{(1)}(\tau)$ is the normalized electric field correlation between the incident and the scattered beam. B is an instrument specific constant, and β is a constant depending on the measurement geometry.

For monodisperse, spherical particles the correlation decay curve can be described by a single exponential term:

$$g^{(1)}(\tau) = \exp(-\Gamma(\tau)) \quad (2.13)$$

The decay rate ($\Gamma(\tau)$) is given by the relation between the diffusion coefficient (D) of the particle and the magnitude of the scattering wave vector (q):

$$\Gamma = Dq^2 \quad (2.14)$$

The magnitude at q is defined as the difference between the incident and the scattered wave vectors, influenced by the refractive index (n) of the solvent, the wavelength of the laser (λ_0) (in vacuum) and the scattering angle (θ):

$$q = \frac{4\pi n}{\lambda_0} \sin\left(\frac{\theta}{2}\right) \quad (2.15)$$

For polydisperse samples the correlation can not be expressed by a simple exponential, but has to be represented as an integral over a distribution at a decay rate $G(\Gamma)$

$$g^{(1)}(\tau) = \int_0^\infty G(\Gamma) \exp(-\Gamma\tau) d\Gamma \quad (2.16)$$

Based on the Stokes - Einstein equation size information about the samples can be obtained since the decay function is related to the center-at-mass diffusion (as definitive property of Brownian motion) of the particles. For spherical particles the relation is as follows:

$$R_h = \frac{k_b T}{6\pi\eta_0 D} \quad (2.17)$$

where R_h is the hydrodynamic radius, η_0 is the relative viscosity of the solvent, k_b is Boltzmann's constant and T is the temperature.

The here obtained R_h can be compared to the molecular sizes defined for polymers in the previous section ($\mathbf{R}$ and R_g). Independently of the applied model, or architecture of the polymer the following relation is always valid:³² $R_h < R_g < \mathbf{R}$.

In addition, light scattering measurements can as well be used to measure rotational time constants for non-spherical molecules in solutions but the theory of scattering from these systems is somewhat more complicated. Macromolecules with rodlike structures can be studied with depolarized light scattering, where the vertically polarized scattered light contains information about the translational and rotational modes, while the depolarized scattered light gives information only about rotational modes.²³²

2.5.2 Atomic force microscopy methods

Over the past decade atomic force microscopy (AFM) has become an essential tool for the characterization and manipulation of surface confined polymer structures at the nanoscale. The AFM experiment is based on attractive/repulsive forces acting between a sharp tip in close proximity of a surface. Due to the high sensitivity of force control, AFM is essentially a non destructive method. The principle of AFM

Figure 2.23: The principle of AFM [Adapted from a reference¹³⁷].

is sketched in Figure 2.23.^{137,163,233–235} Forces are sensed through measuring the deflection of the cantilever by using the optical beam method, in which a laser light is focused on the back of the cantilever and the reflected beam is collected on a position sensitive detector (split photodiode). At the end of the cantilever a sharp

tip is terminated. A piezoelectric scanner controls the position of the sample and moves it with respect to the tip.

Although AFM is originally an imaging technique, which is operated either in contact or in tapping mode, major developments of new AFM based methods evolved the field of AFM force spectroscopy. Nowadays AFM is used for the investigation of the chemical heterogeneity of smooth surfaces, rheological surface/'bulk' properties, as well as for single molecule manipulation. An overview of some of the most basic methods as well as more sophisticated methods is presented here.

Contact and tapping mode

In contact mode AFM a tip attached to the end of a cantilever in mechanical contact is scanned across the sample surface, while the change in cantilever deflection is monitored, through the laser reflection at the back of the cantilever with the optical beam method. A feedback loop maintains a constant deflection of the cantilever at each (x,y) data point by vertically moving the sample. By keeping the cantilever deflection at a preset value, the force between the tip and the sample remains constant.

The force is calculated from Hooke's law in the following way

$$F = kD \quad (2.18)$$

where F is the force, k is the spring constant of the cantilever and D is the cantilever deflection. Force constants usually range from 0.01 to 1.0 N/m, resulting in forces ranging from nN to μ N in an ambient environment.

Figure 2.24: Operation modes of AFM: (a) Contact and (b) tapping modes [Images are reprinted with permission from a reference²³⁶].

Tapping mode AFM operates by scanning a tip attached to the end of an oscil-

lating cantilever across the sample surface. The cantilever is oscillated at or slightly below its resonance frequency with an amplitude ranging typically from 20 to 100 nm. The oscillation of the tip is adjusted such, that the tip lightly 'taps' on the sample surface during scanning, making contact at the bottom of its swing. The feedback loop maintains a constant oscillation amplitude, thus a constant tip-sample interaction is maintained during imaging. If chemical or physical inhomogeneities are present on the sample surface, the phase of the oscillation shifts accordingly. This phase shift is used to distinguish between domains that exhibit different energy dissipation and adhesion, the phase signal at each (x,y) position is captured in a so called phase image. Both of the methods can be operated either in air or in liquid.

Chemical force spectroscopy

Chemical force spectroscopy (CFS) is an AFM-based technique, which allows one to determine distribution map of chemically distinct functional groups on the sample surface. The technique is based on the controlled chemical modification/functionalization of the AFM probe surface, in order to control the interaction between tip and the sample surface. Depending on the exposed functional groups and the medium the following forces can be determined: attractive or repulsive van der Waals forces, forces between dipoles, H-bonding, and electrostatic repulsion.

Figure 2.25: Schematic illustration of surface property mapping experiment, illustrated with a colloid particle probe.

The basic approach during the operation of CFS is to record the force as a func-

tion of the probe vs. piezo position. During a force-distance cycle the probe is first pressed to the sample surface and then retracted. CFS utilizes either a colloid particle or a conventional (sharp) AFM tip attached to the end of the cantilever. The principles of the operation of CFS is sketched in [Figure 2.25](#). The vertical movement of the piezoelectric scanner in a force-distance cycle can be described as shown in [Figure 2.25](#). The cantilever is initially free (position 1). The piezo moves, together with the substrate, towards the cantilever (approach; position 2). The cantilever is then pressed to the substrate, and thus is deflected 'upwards' (positive deflection) (position 3) enabling development of surface interactions between the tip and substrate. While tip and sample are in contact a varying force can be applied to the sample. Upon separation (retraction), due to adhesive interactions (when present) the cantilever is deflected towards the substrate (position 4-5) and a negative deflection is registered. The cantilever is kept at the substrate by the interaction forces. As soon as these acting forces become smaller than the force originated from the spring force of the cantilever that results from its bending, the cantilever rapidly returns to its unperturbed state (position 6).

When using a conventional tip due to the small tip-sample contact area, mapping can be performed down to the sub-50 nm level, while the force-distance curves can be recorded for each pixel of a mesh of pixels. The collected data can be displayed in an image (adhesion map, force-volume imaging) or can be treated statistically to yield a mean value of the pull-off force, represented in a histogram.¹⁵⁸

AFM-based single-molecule force spectroscopy

AFM-based single-molecule force spectroscopy (SMFS) is based on applying and sensing forces through the displacement of a bendable cantilever, with an accuracy of forces around a few pN (see [Figure 2.23](#)). In AFM-SMFS molecular stretching of polymers is performed on a piece of polymer physically (or chemically) tethered between the AFM probe tip and a suitable substrate.

The movement of the piezoelectric scanner during an approach-retract cycle and the corresponding illustration with the cantilever, in AFM-SMFS is illustrated in [Figure 2.26](#). Similarly to CFS, during an experiment, the cantilever is initially free (position 1). Subsequently, the piezo, together with the substrate (containing physisorbed or chemisorbed polymer chains, at a sufficient distance from each other) moves towards the cantilever (approach; position 2). While tip and sample are in contact a varying force can be applied to the sample. The cantilever is then pressed to the substrate and is thus deflected 'upwards' (positive deflection) (position 3) enabling development of interactions between the tip and the polymer strands on the substrate. Upon separation (retraction), the linked macromolecule is first uncoiled (position 4) and stretched, which results in a deflection of the cantilever towards the substrate (position 5). A negative deflection is registered due to chain pulling, and as the chain is entirely stretched, the weakest point of the bridging structure breaks. In other words, the macromolecule desorbs either from the surface or from the tip,

Figure 2.26: Schematic illustration of a single molecule deflection-displacement (piezo position) experiment, the recorded deflection-Z piezo position data and the force-extension curve obtained from deflection- piezo position measurement [Reprinted with permission from the correspondig publisher, from a reference¹³⁷].

thus the cantilever returns to its initial state (position 6).

The recorded cantilever deflection vs. Z piezo position (displacement) ($D - Z_0$) profile (illustrated in Figure 2.26) is converted into force vs. distance (extension) between the AFM tip and the substrate ($F - X$). When the spring constant of the cantilever (k_s) is known the following relationship (Hooke's law) is valid:¹⁷²

$$F = k (D - D_0) \quad (2.19)$$

where D_0 is an offset value (zero separation) at which the tip starts to contact the sample surface. The extension (X) is determined by the combination of the piezo position and the deflection:

$$X = Z_0 + D \quad (2.20)$$

Determination of the zero separation value D_0 is a rather difficult task. In some cases initial forces (attraction or repulsion) are acting when the tip is close to the substrate surface, resulting in additional bending of the cantilever.

Harmonix and peak force tapping

The first compositional maps of heterogeneous polymers and polymer composites based on the variation of mechanical properties were obtained by phase imaging (see AFM tapping mode). The magnitude of the phase signal is related to the amount of

energy dissipated in the material per unit volume, thus it is associated with a material specific property which provides image contrast.^{237,238} harmonix and later on peak force tapping introduced by Veeco (Bruker), held good promise to obtain fast and quantitative surface composition maps. In principle, the harmonix imaging mode is

Figure 2.27: Operation modes of AFM: (a) harmonix and (b) peak force tapping [Images are reprinted with permission from a reference²³⁶].

operated in conventional tapping mode (Figure 2.27). However, it makes use of a specially designed cantilever-tip assembly exhibiting a tip with a lateral offset with respect to the cantilever long axis.²³⁹ The tip is vibrated at its resonance frequency and during intermittent contact, tip-sample interactions twist the cantilever and generate torsional vibrations. This torsional motion is detected, and the collected data is used to generate high speed force-time curves, which are converted to force-distance curves by a mathematical algorithm. From the slope of the force-distance curves obtained in contact the modulus values are calculated. Forces can then be recorded with a microsecond time resolution, allowing for fast imaging (in contrast to point-by-point indentation experiments). The force curves obtained are also used to derive adhesion and dissipation simultaneously, thus in one sweep information about surface modulus, adhesion, energy dissipation and topology is captured. However, harmonix must be calibrated using samples with known surface modulus, which requires well-defined standards. In addition to high imaging speed, the use of ultra sharp carbon tips allows one to obtain a spatial resolution < 10 nm. Development of data evaluation software allows for further analysis providing quantitative distributions of surface modulus values.

Peak force tapping provides mechanical information of sample surfaces, practically

the same as harmonix with the advantage of ease of operation and data analysis. peak force combines the advantages of tapping and contact modes. The sample oscillates (via Z piezo, sample scanner) at a rate of resonance of $f = 2$ kHz (below the resonance of the cantilever), while the cantilever remains at a certain position and the system scans the tip across the surface. The feedback uses the peak force as the control signal to maintain a constant imaging force. Due to the oscillation, individual force - distance curves are collected and the Derjaguin-Muller-Toporov (DMT)¹⁵⁷ modulus as well as adhesion force are calculated in situ.

Both harmonix and peak force tapping successfully resolves material components in complex polymeric systems.^{165,166} The main drawbacks of harmonix are (i) it requires special probes (ii) operation of the system is somewhat complicated and (iii) data analysis requires careful interpretation.

2.5.3 AFM probe modification

The use of chemically modified SPM probe surfaces is well-established in the sensing of chemical/physical forces in contact force measurements. AFM based SMFS and CFS apply and sense forces with an accuracy of a few pN, opening opportunities for the characterization of polymer properties on the nanoscale. Standard AFM tip materials, such as silicon (SiOx) and silicon nitride (Si₃N₄), as well as gold coatings can be used in different liquid environments to measure the interaction between tip and surface in a controlled way. In case of bare probe surfaces the measurable range is limited. In order to extend this approach methods to vary the surface free energy of the tip is required. The desire to obtain complex information of polymer systems stimulated the development of methods and protocols that produce well defined (polymer) modified SPM probe surfaces. This way specific interactions like hydrogen-bonding, hydrophobic or hydrophilic interactions are directly accessible.

One should keep in mind, that handling AFM cantilevers attached to a supporting chip requires careful attention, due to their small size and sensitivity to sudden impact. Therefore modification methods requiring less steps, where the cantilever does not have to be moved from vial to vial, are preferred. For instance, self assembled monolayers on gold surfaces are easily obtained by immersing the substrates in dilute (typically 1-5 mM) solutions of the selected thiol molecule. Vapor deposition of methoxy- or chlorosilane molecules on Si and Si₃N₄ surfaces is another available method. Indeed, functionalization of the probe surface with organic molecules in a well defined manner is based on these well-known surface modification methods.²⁴⁰

The free hydroxyl groups exposed on standard AFM tip materials SiOx and silicon nitride can be directly functionalized using chloro- or ethoxy- substituted silane chemistry (Figure 2.28).²⁴¹ The usually vapor deposited molecules form self-assembled monolayers (SAMs) on the tip surface. This surface functionalization can be enhanced, by crosslinking reactions for the typically used trichloro- or tri- ethoxysilanes, by variations in the number of silanol groups exposed on the tip surface, resulting in a more stable connection. An alternative approach relies on the formation of

Figure 2.28: General route of silicon surface modification with a silane monolayer [Adapted from a reference²⁴⁰].

SAMs on a gold coated tip surfaces.²⁴² Usually, under the Au coating 1 to few nm of Cr or Ti is used as an adhesion promoting layer between Au and the tip. The tip is simply immersed into a solution of a ω -functionalized alkanethiol or disulfide (Figure 2.29). This method results in highly ordered monomolecular layers, which are formed by spontaneous adsorption and organization at the solid-liquid interface. The SAMs used for tip modification, being versatile in terms of surface chemistry (exposed functional groups), are ideal systems for interfacial studies. In addition, several types of functional groups can be incorporated, e.g. $-\text{CH}_3$, $-\text{CF}_3$, $-\text{NH}_2$, $-\text{OH}$, $-\text{COOH}$ and some of these groups may serve as basis for further modifications.

Figure 2.29: Formation of self-assembled monolayers of alkanethiols on gold using (a) thiols or (b) disulfide compounds that show a similar reactivity toward gold surfaces [Adapted from a reference²⁴⁰].

Role of tip modification in AFM-SMFS

Early studies of polymeric materials regarding their behavior at the nanoscale upon applying forces mainly involved SMFS, where mechanical properties of single chains were measured. In experiments the polymer should bridge a substrate and an AFM tip, being (covalently or physically) bound to either (or both) of them. The facing surfaces are separated from each other thereby pulling the polymer chains.

Polymer chains attached to the planar surface may connect to the AFM tip as

it approaches the substrate. Some chains may adsorb, establishing the connection required for the experiment. This method is often used, but the main disadvantage of this 'fishing' for the chains is the low success-rate. Successful attachment to the tip surface depends on the strength of the bond that is developed during the short time period when the tip is in contact with the surface. The strength of physical bonds between the tip and polymer chains are in the order²⁴³ of 50 -2000 pN, depending on the properties of the tip surface. In case of unmodified probes such strong bonds strengths are difficult to establish. Therefore, sometimes it is more convenient to attach polymer chains to the tip surface rather than to the planar surface, as there is more chance for adsorption to the opposing surface. Another advantage of this attachment method is that a better control of the amount of the material is achieved, hence if of interest, detachment forces of 'single' chains from the modified opposing surfaces are easily studied.

Tip modification methods have developed rapidly and nowadays there are several options for controlling the amount of grafted chains independently on gold coated or Si_3N_4 probes. The use of self assembled monolayer deposition on the tip surface allows one to establish a stable layer for further chemical modification to attach polymer chains. Some of the modification routines take advantage of the chemical properties of the polymer chain itself, e.g. polyamines are easy attachable to epoxy groups,¹⁴⁵ or polyacides²⁴⁴ to hydroxy groups of the previously deposited monolayer. In the referred examples stable polymer layers were formed on the tip surface showing high stability under various environmental conditions. This way of attachment the density of the polymer chains are not controlled, and the attachment points among the molecules are not well defined either, in terms of the amount of chains and the number of segment connected to the surface.

End tethered polymer chains bound to the probe surface offer the opportunity to study entropic/elastic properties of single polymer chains.¹³⁷ This knowledge was successfully implemented in the development of targeted assembling of DNA molecules^{225,245–248} and design of TREC imaging²⁴⁹ and 'biosensors' based on polymeric linker molecules supporting of various biological complementary molecules (Figure 2.30).

One of the most commonly used routines for the modification of Si_3N_4 probes involves deposition of an amine terminated silane monolayer, followed by coupling polymer chains to the amine groups. The Si-O bond forms a relatively stable connection between the polymer chain and the tip surface, and the amine functionality enables one to use several chemical coupling methods such as EDC/NHS^{250–252} or thiol-succinimide coupling (Figure 2.30).²⁵³

There are numerous applications^{136,249,254–259} where poly(ethylene glycol) (PEG) is used as a linker between a substrate and the functional groups of interest, due to its outstanding properties (*i.e.* PEG is easy to obtain in monodisperse, low molar mass form, with various end-functionalities). Monolayers of PEG with thiol or disulfide functionality can be formed on the surface of gold coated AFM tips, either modified with the molecules of interest²⁵⁸ or modified afterwards.^{136,254,260–262} Gold coated

Figure 2.30: AFM tip functionalization with aldehyde-PEG-NHS as compared to acetal-PEG-NHS. Loop formation between adjacent amino groups can occur with aldehyde-PEG-NHS (a); it is largely suppressed at high concentrations of aldehyde-PEG-NHS (b). No loop formation occurs with acetal-PEG-NHS (c), since the acetal is converted into aldehyde only after derivatization of all NH_2 groups on the AFM tip (d). Lysine residues of proteins can be coupled to terminal benzaldehyde functions (e) [Reprinted with permission from a reference.²⁵⁰ Copyright (2012), American Chemical Society].

probes are appealing for modification with biomolecules, since most of the proteins contain cysteine in their sequence,²⁶³ but similarly cysteine can be introduced as the functional end group of synthetic polymers.¹⁴⁷

The targeted application may require one to control the grafting density of the polymer chains. For instance, the attached polymer can serve as a basis to which sensor molecules are connected. At the same time, an excess of polymer can be applied to passivate the surface, preventing non-specific binding.^{251,252} In other cases it is crucial to have single polymer chains at the end of the tip,²⁶⁴ but attaching single chains to the end of a tip is rather challenging. One may fabricate mixed monolayers to lower the number of grafted polymer chains. This can be achieved

Figure 2.31: (a) Design of a molecular tripod tip A for ligand receptor AFM force spectroscopy and (b) comparison with a molecular monopod tip B system. Because of the wider base of the molecular tripod A, the density of the molecules on the modified AFM tip surface is lower than with monopod B (see (b)), enabling a higher probability of single molecule measurements. The attachment of the molecular tripod A through the three disulfide legs provides more stable immobilization of the molecule, giving more robust chemically modified probes. [Reprinted with permission from a reference.²⁵⁵ Copyright (2012), American Chemical Society].

either by applying a mixed solution of surface-reactive components^{140,265} or by attachment of molecules that are partially modified afterwards, where the unmodified ones will serve as a surface-bound diluent.^{250,254} The space taken by the surface-anchored polymer²⁵⁵ can be tuned by utilizing multiple attachment points, (e.g. a tripod molecule, Figure 2.31) thus reducing the number of molecules on the surface while in addition providing better control over the stability of the layer.

A rather unique way of surface modification, established by Duwez *et al.*^{266–268} is electrografting of the polymer chain. Well-defined polymer chains were grown from gold coated tip surfaces by immersing the AFM probe in the solution of the electroactive monomer and applying an electric potential on it. This method resulted in a low grafting density and allowed the authors to deliver single chains to a targeted place.²⁶⁷ Even on Si_3N_4 tip it is possible to electrograft polymer chains, due to defect sites in the Si_3N_x layer, and by varying the monomer concentration the graft density was tuned, allowing further modification of the polymer chains.²⁶⁹

A dense layer of end grafted polymers can be formed on the tip surface after deposition of appropriate initiator molecules.^{176,270} Growing polymer brushes on a tip surface however is a rather difficult task from the point of view of handling the AFM probes, since most of the polymerization methods require inert atmosphere, and multiple washing and cleaning steps.^{35,37}

As the above listed examples have show, the main objective of tip modification by polymers is to obtain a well defined connection between tip surface and a desired

number of polymer chains, most preferably attached via their chain end. Besides these applications, in some cases tip surfaces are modified by coating with certain polymers to obtain an isolating layer²⁷¹ on it, to study interaction forces of polymer film surfaces with the modelling surfaces under varying environmental conditions²⁷² and to probe specific affinity^{273,274} towards other surfaces by chemical force titration.

2.6 Conclusion

Responsive polymers as well as multi responsive materials that react reversibly to external stimuli have attracted particular attention over the last decades. The recent significant progress in the synthesis of well-defined macromolecular architectures and the polymerization of highly functional building blocks using precisely controlled polymerization methods has led to the construction of numerous examples of responsive architectures. These polymeric materials appear in the form of, among others, linear or graft copolymers and polymer surfaces or interfaces. Understanding the materials' physical-chemical properties is required for the implementation in useful applications, therefore evaluation of their behavior must start at the single chain level. The extended development of AFM methods and AFM based spectroscopy techniques are a great contribution to the evaluation of stimulus responsive properties at the nanoscale.

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Chapter 3

Nanomechanical properties of polymer brushes by colloidal AFM probes *

Nanomechanical properties of end grafted polymer layers were studied by AFM based colloidal probe compression measurements. Zwitterionic poly(sulfobetaine methacrylate) (PSBMA) brushes were grafted from planar Si surfaces and poly(methyl methacrylic acid) (PMAA) brushes were grown on colloidal probes by surface initiated atom transfer radical polymerization. PMAA brushes were further modified with adhesion promoting arginyl-glycyl-aspartic acid (RGD) peptide sequences. AFM Force - distance curves were obtained for systems where the polymer brushes were probed on unmodified surfaces or face to each other. For each system the grafting density of the polymer brush was determined applying a 'box' like polymer brush model based on the theory by de Gennes. 'Average' grafting densities were calculated in cases when two polymer brushes faced each other: RGD functionalized PMAA or PMAA against PSBMA. For our systems the values for the grafting density was between 0.04 and 0.11 chains/nm². The measured approach force-distance curves were fitted according to the Hertz model and the apparent Young's modulus was determined for all measurements being in a range of around 250 kPa at physiological conditions.

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3.1 Introduction

End grafted polymer layers covalently attached to solid supports are widely used to modify substrate surfaces.^{1–3} Depending on the ratio of the average distance between the attachment points to the unperturbed coil dimensions (twice radius of gyration) the grafted chains can possess various conformations if swollen by a solvent.^{4,5} For values of this ratio smaller than ca. 0.05, the chains are essentially unperturbed, while for high densities of the anchoring sites they can adopt strongly extended conformations. Such densely grafted systems are referred to as polymer brushes. These can either be applied as homogeneous films (with or without gradients), or in a patterned form.⁶

The two most important structural parameters of a polymer brush thus encompass the grafting density and the length (or length distribution) of the chains. These parameters are not only important for the understanding of the physical behavior and properties of the brush, but also play a crucial role in applications.^{7,8} Yet the precise and accurate determination of these parameters remains a difficult task.⁹ The Alexander-de Gennes model provides in principle a theoretical framework and allows one, in a first approximation, to relate the height of a brush in a solvent with the grafting density and the degree of polymerization.^{10,11} Here we demonstrate that by measuring brush-brush interaction forces under compression the grafting densities can be estimated by a relatively simple process. In this process we make use of the results of de Gennes¹² which relate compression forces to brush thickness, substrate separation and grafting density.

Elastic properties, such as compression modulus, of brushes are determined by the chain length, the grafting density, as well as the primary structure of the grafted chains. Knowledge of compressive brush elasticity is relevant for numerous applications such as in cell adhesion,¹³ brush biomembrane supports and bending rigidity,^{14,15} microfluidics,¹⁶ etc. In this work we used atomic force microscopy (AFM) based colloidal probe force measurements to assess the compressive response of brushes for the estimation of grafting density values, as well as for the determination of the values of compression moduli.

Recent progress in controlled free radical polymerizations allows one to prepare polymer brushes using a broad variety of monomers.¹⁷ Due to the quasi living character of many of these polymerizations, block copolymer structures and other controlled morphologies can be prepared. The brush chains can feature neutral, or charged, macromolecules, as well as zwitterionic polymers. Here we chose two polymer systems for 'grafting from' growth of brushes, *i.e.* poly(methacrylic acid) (PMAA) and poly(sulfobetaine methacrylate) (PSBMA). Both brushes were obtained by atom transfer radical polymerization (ATRP), which is a widely applied, controlled free radical polymerization method to obtain brushes by the 'grafting from'¹⁸ approach. This rather simple process has gained popularity owing to its controllability and reproducibility. While PMAA forms polyions with a surface pKa of 6.5,¹⁹ PSBMA features zwitterionic systems. The brushes were investigated in buffer salt solutions

with deprotonated PMAA chains and with a fixed ionic strength that is typical for physiological conditions.

3.1.1 Nanomechanical response of polymer brushes by colloidal probe AFM: Background

Several ways exist to determine the force response of adsorbed, or grafted, polymer layers, including the surface forces apparatus (SFA),^{20,21} the use of AFM with 'regular' sharp AFM probes, or AFM with colloidal probes.

Force measurement based on AFM is a widely used technique to probe interactions between surfaces.²² Values of typical AFM probe tip radii are in the range of several tens of nanometers, thus they can easily penetrate into the brush due to high local pressure.²³ In order to circumvent this problem, spherical colloid particles with radii on the order of micrometers can be used as probes attached to AFM cantilevers.²⁴ Such colloidal probe AFMs benefit from the knowledge of the geometry of the probe, and distribute the pressure on the sample surface over a larger contact area, while the force sensitivity of the AFM is maintained. Thus this method is very well suited to characterize the mechanical response of brushes in compression to obtain grafting density and modulus values.

The theoretical basis for all techniques to determine the force response of brushes has been provided by de Gennes.¹² According to de Gennes, when two parallel surfaces covered with brushes approach each other, the forces acting between them depend on the grafting density of the polymers and on the brush thickness. The thermal movement of a polymer chain is limited by the approach of another surface, and upon approach in contact the entropy of individual polymers decreases. In addition, the 'concentration' of segments in the gap increases. This leads to an increased osmotic pressure. Following the original de Gennes treatment the resulting force between a spherical body and a planar surface, both covered with a brush, for distances smaller than twice the brush thickness can be described with the following equation:^{25–27}

$$\frac{F}{R} = \frac{8k_b T L}{35} \Gamma^{3/2} \left[7 \left(\frac{d}{2L} \right)^{-5/4} + 5 \left(\frac{d}{2L} \right)^{7/4} - 12 \right] \quad (3.1)$$

In case of an asymmetric arrangement, when only one of the surfaces bears a polymer brush the equation is modified as follows:

$$\frac{F}{R} = \frac{2k_b T L}{35} \Gamma^{3/2} \left[7 \left(\frac{d}{2L} \right)^{-5/4} + 5 \left(\frac{d}{2L} \right)^{7/4} - 12 \right] \quad (3.2)$$

Here, F is the force acting between the surfaces, upon compression, R is the radius of the curved surface (in our case of the colloidal probe, $1 \mu\text{m}$), Γ is the number of bound polymer chains per unit area (grafting density), k_b and T are the Boltzmann

constant and temperature; L is the thickness of the brush on each sides and d is the distance between the surfaces.

To measure the stiffness of the polymer brushes in compression in a quantitative way, the value of the apparent Young's modulus of the polymer brush can be determined based on the Hertz model of elastic contacts.²⁸

Upon compression of two contacting surfaces the slope of the force - indentation depth curves is related to the combined modulus of the contacting bodies. The indentation depth is the difference between the separation distance that would be obtained on a hard surface compared to the separation distance obtained for a soft sample. The deformation (δ) of the surface can be obtained from the approach part of the deflection – distance curves, according to:

$$\delta = |Z - Z_0| - (D - D_0) \quad (3.3)$$

where Z denotes the piezo - extension, D is the value of deflection of the cantilever, Z_0 and D_0 are values of an offset at which the tip starts to contact the sample surface. The deflection of the cantilever is converted to the load (F) applied onto the polymer brush by applying Hooke's law:

$$F = k(D - D_0) \quad (3.4)$$

where k represents the spring constant of the AFM cantilever. In order to determine the values of the apparent Young's modulus of the polymer brush the Hertz equation is used:

$$F = \frac{4\sqrt{r_{tip}}}{3(1-\nu^2)} E \delta^{3/2} \quad (3.5)$$

where r_{tip} is the radius of the colloidal probe, ν is the Poisson ratio (0.5) and E is the apparent Young's modulus of the polymer brush.

Colloidal probe measurements allow us to investigate nano-mechanical/ rheological properties either of the flat substrate or the colloidal bead attached to the cantilever.^{29–33}

3.1.2 Choice of systems

Planar surfaces featuring polymer brushes studied here were based on PSBMA. This is a widely studied polymer brush due to its anti-biofouling property.^{34,35} In liquid environment a unique hydration layer is formed at PSBMA brushes that is responsible for its protein repellent behaviour.^{15,36,37} This brush as a planar system was studied with colloidal probes, either surface modified or unmodified. The surface of colloidal probes can be modified similar to AFM conventional tips by chemical surface modifications.³⁸ This can be for example performed by covalently bound polymerization initiator to cover the tip by a polymer brush using the 'grafting from' approach.

Different types of colloid probes are widely available to support all kinds of chemical modifications. In our study we used gold colloid particles, therefore the colloid

surface could be modified and eventually brushes grafted, via gold-thiol chemistry. A typical AFM colloidal probe experiment, (capturing) PMAA brushes (with RGD adhesion proteins attached) at the colloidal probe surface (mounted on a Si_3N_4 cantilever) and PSBMA brushes at planar substrates, is schematically shown in Figure 3.1. In the experiments, described in this Chapter, colloidal probes covered with PMAA and PMAA-RGD, as well as without brush, were used to probe planar PSBMA brushes and uncoated silicon surfaces.

Figure 3.1: Scheme of the performed measurement, RGD functionalized PMAA brush on the colloid probe and PSBMA on the flat surface.

The environment was chosen close to physiological conditions which also ensured the negligible adhesion acting between the polymer surfaces. PMAA was chosen as a brush for colloid modification as it allows easy modification by the adhesion promoting RGD (L-arginyl-glycyl-L-aspartic acid) peptide sequence.³⁹ RGD functionalization has relevance for studies of interaction forces between this model biological adhesion layer with the zwitterionic brush. Results on biological adhesion tests will be published elsewhere.

3.2 Results and Discussion

As mentioned in section 3.1.2, various combinations of brushes at the colloidal probe and planar surfaces were used. For easy identification of the systems measured we summarize the performed experiments in Table 3.1.

3.2.1 Determination of grafting density

Force–distance approach curves obtained for systems 1 and 2 (see Table 3.1) are shown in Figure 3.2a in comparison with system 3 as a reference. The corresponding F/R plots as a function of separation distance (see Equation 3.1 and 3.2) are

Table 3.1: Summary of the samples and experiments.

Experiment	tip	Surface
1	PMAA-RGD	PSBMA
2	PMAA	PSBMA
3	unmodified gold	PSBMA
4	PMAA-RGD	Si
5	PMAA	Si
6	unmodified gold	Si

captured in Figure 3.2b. Figure 3.3a displays the systems in asymmetrical cases *i.e.* when the probe surface is covered by polymer brush (experiments 4 and 5). In Figure 3.3b the corresponding F/R plot is shown. Experiment 6, encompassing compression of unmodified gold against Si substrate is also shown as a reference in Figure 3.3a. F/R–distance plots were fitted according to Equation 3.1 for the symmetrical case and by Equation 3.2 for the asymmetrical systems, as displayed in Figure 3.2b and Figure 3.3b.

As the measurement data for interacting brushes show, with decreasing separation repulsion forces dominate (see Figure 3.2a and 3.3a), which originate in steric and electrostatic repulsions. In Figure 3.2a result for an 'asymmetric' arrangement (PSBMA brush on substrate probed with unmodified Au colloid) is also provided. Figure 3.3a captures, as 'blank', force acting between unmodified Si substrate and unmodified Au colloid probe. As expected, compression of the hard substrates provides a straight line (Hooke's law) with a slope close to 90° corresponding to high values of moduli. (We note that the slope also reflects piezo creep contribution of the AFM scanner and furthermore due to the adhesive used to attach the colloid to the cantilever, thus we do not provide any modulus estimation).

Fits to Equation 3.1 and 3.2 and the corresponding data sets (symmetrical and asymmetric cases, respectively) are shown in Figure 3.2b and 3.3b, using log-log plots.

Although we used Equation 3.1 and 3.2 for the fits using de Gennes 'box' model, we mention that Block et al.²⁵ performed AFM force–distance curves that were fitted according to two different theories based on the profile assumption of the brush. The brush can be regarded to possess a box like structure (de Gennes) or a more complicated, parabolic profile, *i.e.* according to the Milner-Witten-Cates (MWC) theory.⁴⁰ In their work it was concluded that measuring at salt concentrations above 100 mM the fitted curves are identical to each other. Therefore, in our case fitting either of the models should not show any relevant differences. We have chosen to apply the de Gennes theory, due to its simplicity.

It is important to point out, that in our case, zero surface separation can never be reached due to the finite brush thickness. The theoretical force profile goes to infinity

Figure 3.2: (a) Approach curves on PSBMA brush with polymer coated and unmodified probe (b) log-log plot with corresponding fitting according to equation 3.1 and 3.2 (note that the curves are representative examples, not averaged).

Figure 3.3: (a) Approach curves on Si surface (b) log-log plot with corresponding fitting according to equation 3.2 (note that the curves are representative examples, not averaged).

as the surface separation reaches zero, and hence there will always be a displacement offset (z_{offs}) between the surface separation calculated from the data, Z (the measured piezo-tip displacement) and the true surface separation $d = Z + z_{offs}$. While we apply this equation for the fitting we have to assume that the brush is compressed fully, therefore we analyzed the data only where the applied load was at least 3 nN. According to this assumption the fitting gives us a value for the brush thickness that equals to the thickness of the compressed brush, and the offset value is compensating for it (see Supporting Information).

In each measurement set 20–30 curves were chosen. For all cases studied, we

found values for the grafting density between 0.04 and 0.11 chains/nm², namely 0.053 ± 0.010 for the PMAA brush grafted from colloid tip surface (experiment 5), 0.085 ± 0.027 chains/nm² for the RGD motif functionalized PMAA brush grafted surface (experiment 4), and for the Si grafted PSBMA (experiment 3) 0.11 chains/nm² was found (see also Table 3.2). In these cases the brushes were compressed by unmodified surfaces previously termed as 'asymmetric'.

When two polymer brushes face each other (referred to as 'symmetric') then an 'average' grafting density can be calculated. Although in the cases we studied the primary chemical structures of the polymers are different, these and most notably electrostatic charging apparently do not significantly influence the Γ values. The overall ionic strength of the solution is 170 mM, which results in a value for the Debye length of 0.82 nm. Note that the Bjerrum length where the screening becomes more pronounced to this scenario, is 0.7 nm, so we are rather close to it.

Compressing the polymer brushes gives an average grafting density value of 0.042 ± 0.003 chains/nm² in case of PMAA facing a PSBMA brush (experiment 2), and $\Gamma = 0.067 \pm 0.001$ chains/nm² for RGD functionalized PMAA and PSBMA (experiment 1). According to Equation 3.1 and de Gennes theory, the force acting between the two polymer surfaces is due to entropy and osmotic pressure contributes. In case of the RGD tripeptide modified surfaces we expect that the local density as well as the osmotic pressure are higher than in case of the original PMAA brush.

We expected that due to the additional space requirement within the brush by attaching RGD, the osmotic pressure would increase resulting in an enhanced compression. We attribute the apparent increase of the effective grafting density values of the RGD modified PMAA to this effect (see Table 3.2).

The fitted values for all cases show the same trend. In case of the PMAA the grafting density seems to be lower in all surfaces than for the RGD modified one.

Table 3.2: Grafting density values for different systems, in chains/nm².

	Unmodified Si	PSBMA brush
Unmodified gold	-	0.11
PMAA brush	0.053 ± 0.010	0.042 ± 0.003
PMAA-RGD brush	0.085 ± 0.027	0.069 ± 0.001

3.2.2 Stiffness of the polymer brushes

The mechanical properties of the different polymer brushes under compression were evaluated from AFM colloid probe force–distance curves. The fundamentals of the experiments are described earlier.

To measure the stiffness of the polymer brushes in a quantitative way, the apparent Young's modulus of the polymer brush was determined from the recorded curves

based on the Hertz model. Typical approach section of force–distance curves for the different systems are shown in Figures 3.2a and 3.3a.

Considering the slope of the approach curves the apparent Young’s modulus was calculated for PSBMA brush from measurements by a gold bead against the polymer layer. Data for PMAA brush and RGD functionalized PMAA-brush were obtained from measurements with modified colloid probes against a Si substrate. The initial section of the compression part up to around 1 nN force was fitted with the Hertz model. Figure 3.4a shows the histograms of the collected data and the corresponding Gaussian fit to determine the average mean value of the Young’s modulus. Results obtained are summarized in Table 3.3.

Figure 3.4: Apparent Young’s modulus (a) for PSBMA brush grafted from Si substrate, PMAA and PMAA-RGD grafted from AFM probe, measured against Si substrate and (b) for brush-brush interactions.

Table 3.3: Apperent Young’s modulus values for different systems, in kPa.

	Unmodified Si	PSBMA brush
PMAA brush	260 ± 80	210 ± 60
PMAA-RGD brush	240 ± 60	220 ± 40
Unmodified gold	-	240 ± 8

PSBMA polymer brush systems grafted from Si substrates (experiment 3) showed a Young’s modulus of 240 ± 8 kPa, calculated as an arithmetic mean value of the measured distribution.

Colloid AFM probes were modified by grafting PMAA from the surface and were further functionalized with the RGD tripeptide motif. The values of apparent Young’s moduli were calculated to be 260 ± 80 kPa and 240 ± 60 kPa for PMAA brush (experiment 5) and for PMAA-RGD brush (experiment 4), respectively, using equation

3.5. We applied the assumption here that it is irrelevant in terms of the modulus data whether the polymer is anchored to the flat surface or to the curved one.

An apparent stiffness can be calculated by compressing the different polymer brushes to each other (see Figure 3.4b). When the AFM tip approaches the surface and the polymers are in contact we can treat them as one polymer layer between two stiff surfaces. Applying the Hertz model, the stiffness was calculated for these mixed systems as well. The values were found to be in the same order as for the separate polymer brushes: 210 ± 60 kPa and 220 ± 40 kPa for PMAA grafted probes (experiment 2) and RGD functionalized surfaces (experiment 1) on the PSBMA brush, respectively.

It is remarkable that there is a little deviation in the compression modulus for each sample measured. The measured range for the value is in agreement with the literature previously reported⁴¹ for PNIPAM polymer brushes grafted from Si. For polybutylacrylate brushes⁴² with 10 times higher grafting density compared to our system, the value was in the range of 30–80 MPa. In our case the stiffness of the polymer chains is determined by the electrostatic interactions among the polymer chains and primarily therefore influenced by the medium. As mentioned, due to the experimental conditions, the Debye length $\kappa^{-1} = 0.82$ nm and the Bjerrum length where the screening becomes more dominant, is 0.7 nm. Yet electrostatic interactions are important to determine the stiffness of the polymer brushes. We mention that throughout this measurement we assume that the stress field caused by the colloidal compression decays off within the brush as its chains become longer, *i.e.* substrate effects can be neglected. Considering the differences between the two polymers (PNIPAM and zwitterionic charged systems), one expects a higher stiffness in case of the zwitterionic brush, assuming similar grafting density (which likely holds as the initiator deposition was carried out the same way). The influence of the grafting density on the stiffness is discussed by Sui *et al.*⁴¹. This supports our argument as discussed above, *i.e.* that similarly structured polymer brushes would have similar elastic properties in compression.

3.2.3 Adhesion properties of the polymer brushes

It is of importance to emphasize the interaction forces between the contacting surfaces, upon approach and retraction. As previously discussed, repulsion forces, which originate from steric and electrostatic repulsions, are dominating with decreasing separation (approaching, see Figure 3.2a and 3.3a). Figure 3.5 shows the distribution of the measured pull-off forces upon retracting the brushes in reference to the unmodified probe. Neither PMAA nor PMAA-RGD modified colloidal probes showed significant adhesion to the PSBMA grafted silicon substrate. Adhesion forces observed between the different surfaces were between 0 and 1.5 nN. In case of a colloidal probe, the contact area is larger compared to a conventional AFM tip and therefore more sensitive to attractive forces compared to conventional sharp AFM tip; hence the measured forces have larger values. In most cases no adhesion was observed,

Figure 3.5: Distribution of adhesion forces on PSBMA brush in PBS buffer with polymer coated and unmodified probe.

as shown in the frequency histograms. An unmodified gold probe has no particular adhesion to the PSBMA graft surface either. When different loads were applied from 1-10 nN, no tendency to increase the adhesion force was observed. Measured pull-off forces originated from the surface roughness of the gold probe.

Figure 3.6: Distribution of adhesion forces on NH_2 functionalized surfaces in PBS buffer with different ionic strength (a) PMAA (b) RGD modified probe.

Additionally, experimental data were collected to confirm successful modification of the colloidal AFM probe and also formation of the PMAA-RGD conjugate. Force measurements alone, conducted using a representative probe and a well characterized modified substrate, can give important indications of a successful AFM probe modi-

fication. As mentioned in the previous sections, the mechanical response of PMAA and PMAA-RGD brush-modified colloid probe as well as Si surface-grafted PSBMA brushes were analysed. To gather further experimental evidence of successful colloidal probe modification, adhesion measurements were carried out on cysteamine modified gold substrates under variation of the ionic strength (NaCl concentration) of the buffer solution. In [Figure 3.6](#) the distributions of pull-off forces obtained with PMAA and PMAA-RGD modified colloidal probes on cysteamine-modified gold substrates are shown. Both PMAA and PMAA-RGD interact mainly electrostatically with the cysteamine modified substrate. At high ionic strength (1 M NaCl), PMAA and PMAA-RGD both showed low adhesion to cysteamine-modified gold substrates, providing similar pull-off force values of 0.22 ± 0.07 and 0.219 ± 0.08 nN for PMAA and PMAA-RGD, respectively. A different situation was observed for 500 mM NaCl, where forces of 0.24 ± 0.09 nN were recorded for PMAA while PMAA-RGD forces were threefold higher at 0.76 ± 0.30 nN. Even more significant was the difference in observed pull-off forces at the lowest ionic strength of 150 mM NaCl. Here, the PMAA-RGD colloidal probe showed a fivefold higher pull-off force of 2.57 ± 0.99 nN compared to the PMAA-modified probe (0.52 ± 0.22 nN). This clearly demonstrates the influence of electrostatic screening; pull-off forces significantly decrease with increasing electrolyte concentration. At pH 7.4 the cysteamine is protonated and positively charged while PMAA and PMAA-RGD will significantly differ in their electrostatic nature.

3.3 Summary and Conclusion

In this Chapter we showed a method to determine grafting density and nanomechanical properties of end grafted polymer layers by AFM based colloidal probe compression measurements. As a planar surface, zwitterionic PSBMA brush was grafted from Si and PMAA brushes were grown on gold colloidal probes by SI-ATRP. PMAA brush was further modified with adhesion promoting RGD peptide sequences. The polymer brushes were probed against unmodified surfaces and against each other. For each system the grafting density was determined, applying a 'box' like polymer brush model based on the theory by de Gennes. Si grafted PSBMA had a grafting density (Γ) of 0.11 chains/nm², 0.053 ± 0.010 chains/nm² was obtained for the PMAA brush grafted from colloidal tip surface and 0.085 ± 0.027 chains/nm² for the RGD motif functionalized PMAA brush grafted surface. The values of 'average' grafting density were calculated in cases when two polymer brushes face each other: $\Gamma = 0.042 \pm 0.003$ chains/nm² in case of PMAA facing a PSBMA brush, and $\Gamma = 0.067 \pm 0.001$ chains/nm² for RGD functionalized PMAA against PSBMA. The measured approach force–distance curves were fitted according to the Hertz model and the apparent Young's modulus was determined for all measurement sets. The obtained values were around 250 kPa.

Polymer brushes bearing similar charge density and grafting density show similar

elastic properties upon compression. Further measurements will be focused on the study of the influence of different environmental conditions on the stiffness of the polymer brushes, for example varying the ionic strength, the pH of the medium and the temperature.

3.4 Experimental section

Chemicals were purchased from Sigma-Aldrich (The Netherlands) and used as received if not stated otherwise. Organic solvents were purchased from Biosolve (Valkenswaard, The Netherlands), hydrogen peroxide and ethanol was purchased from Merck (The Netherlands). CuBr was stirred overnight with 98% acetic acid solution, filtered, washed with methanol and dried under vacuum. Deionized water from a MilliQ Advantage A 10 purification system (Millipore, Billerica, Ma, USA) was used.

Preparation of PSBMA surfaces¹⁹

Initiator molecules were immobilized on freshly cleaned silicon substrates by vapor phase deposition overnight under vacuum. Prior to the deposition, substrates were cleaned by sonication in ethanol (15 min.) and also in pure water for 15 min. Subsequently, the substrates were cleaned in piranha solution (7:3 v/v% mixture of H₂SO₄ (95-98%) and H₂O₂ (30%)) for a few minutes, rinsed extensively with water, ethanol, and dried (piranha solution reacts strongly with organic compounds and should be handled with extreme caution). After initiator deposition, substrates were sonicated in ethanol for 2 min, rinsed with ethanol and dried under nitrogen gas. Initiator-immobilized substrates were immediately used for ATRP. For polymerization the initiator-covered substrates were placed in dry vials and purged with argon for 2 h. The monomer, [2-(methacryloxy)ethyl]-dimethyl-(3-sulfopropyl)-ammonium hydroxide (SBMA) (5.58 g, 20 mmol) was dissolved in the ATRP medium (40 mL water/methanol mixture with ratio 1:4) and the solution was degassed for 2 h. CuBr (286 mg, 2 mmol), CuBr₂ (53 mg, 0.24 mmol), and 2,2-bipyridine (626 mg, 4 mmol) were added to a flask equipped with a magnetic stirring bar, and deoxygenized by 3 vacuum-Ar backfill cycles. The degassed monomer solution was transferred into the flask and stirred under Ar for a further 15 min until a clear brown solution was observed. Afterwards, the polymerization mixture was injected into each reaction vial, adding enough solution to submerge each sample completely. The polymerization was conducted for 1 h at room temperature, after which the samples were removed from the vials and washed with Milli-Q water. After a brief sonication in EDTA solution (0.1 M, pH 7) to extract residual copper ions, the substrates were immersed in water overnight to remove any physisorbed polymer. Finally, the substrates were rinsed with ethanol and dried under nitrogen gas.

Modification of AFM colloidal probe surface and gold surfaces

Immobilization of SAMs on gold coated surface

Gold coated substrates (100 nm gold evaporated on Si wafer having a 10 nm Cr adhesion layer) and gold colloidal probes (SQube, CP-FM-Au, Nano and More, Spilburg, Germany) were cleaned first with chloroform, piranha solution, subsequently rinsed with Milli-Q water,

ethanol and immersed in the monolayer solution for overnight. (We note that prolonged exposure to piranha results in detachment of the colloidal particle.) Monolayer solutions were prepared in degassed chloroform solution with 2-bromo-2-methyl-propionic acid and 11-[11-(2-bromo-2-methyl-propionyloxy)-undecyldisulfanyl]-undecyl ester (20 ml with the concentration of 0.2 mM)¹⁸ and degassed ethanolic solutions of cysteamine (~ 1 nM).

SI-ATRP of PMAA from gold coated substrates and gold colloidal probes

PMAA brushes were grafted from SAM modified gold substrates and AFM probes by SI-ATRP. The initiator-covered substrates were placed in dry vials and purged with argon for half an hour. The monomer sodium methacrylate (3.0 g, 0.027 mol) was dissolved in the ATRP medium (10 ml water/methanol mixture with ratio 1:1) and deoxygenated by bubbling for half an hour. Concurrently, 2,2-bipyridine (31 mg, 0.2 mmol) and CuBr (28 mg, 0.2 mmol) were added to a flask equipped with a magnetic stirrer bar and deoxygenized by 3 vacuum - Ar backfill cycles. The degassed monomer solution was transferred into the flask and stirred under Ar for a further 15 min until a clear brown solution was observed. The polymerization mixture was injected into each reaction vial in a way that each sample submerged completely in the solution. The polymerization was conducted for 3 hours at room temperature, and the reaction was terminated by exposing the solutions to air. The samples were subsequently removed from the vials and washed with MilliQ water. PMAA brush modified surfaces were kept in 1 M EDTA (pH=7.4) solution overnight to remove all the copper.

RGD functionalization of the PMAA modified gold colloid probes

To activate the carboxylic acid groups of surface-immobilized PMAA,³⁹ active ester chemistry was applied using an EDC/NHS solution: (EDC: *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide) 38 mg/ml and NHS: *N*-hydroxysuccinimide 6 mg/ml in phosphate buffer (pH 7.4, 0.1 M NaCl). Colloid probes and substrates were submerged in the activation solution for 1 h. After removing the tips and substrates from the activation solution, they were rinsed with buffer solution, and subsequently immersed in the RGD (L-arginyl-glycyl-L-aspartic acid) tripeptide solution (2 mM in phosphate buffer) overnight. Finally, the PMAA modified and RGD functionalized AFM tips were rinsed gently with phosphate buffer solution and kept in the same solution. The flat substrates were kept under N₂ atmosphere.

AFM measurements

The AFM measurements were performed on a Picoforce AFM using a NanoScope IVa controller, a Picoforce vertical engage scanner and a liquid cell (Bruker/Veeco/Digital Instruments, Santa Barbara, CA). 2 μ m in diameter, gold colloidal probes were functionalized as described earlier. The cantilever spring constants (between 0.05-0.14 N/m) were determined in liquid via the thermal tune method.⁴³ The measurements were carried out in liquid environment applying a phosphate buffered saline (PBS buffer) at pH=7.4 at 150 mM NaCl concentration (B. Braun Melsungen AG, Melsungen, Germany). 500 mM and 1M NaCl buffer solutions were prepared by supplying additional NaCl to the starting buffer solution. Surfaces were subsequently measured with the same colloidal probe: first the polymer brush, next the Si reference surface, followed by the amine-terminated monolayer surface. Here the salt concentration was increased and finally the polymer surface was measured at 1 M

NaCl concentration to avoid errors due to ions remaining in the polymer brush grafted onto the probe surface. The ramp rate was kept at 0.5 Hz, therefore the forward and retract velocity was 1 $\mu\text{m/s}$. The applied load was varied between 1 nN and 12 nN triggered by the deflection of the cantilever. Force distance curves were analyzed with PUNIAS software (<http://punias.voila.net/>) to obtain the apparent Young's modulus data.

3.5 References

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Chapter 4

Is there a molecular signature of the LCST of single PNIPAM chains as measured by AFM force spectroscopy?*

The molecular stretching behavior of temperature responsive poly(N-isopropylacrylamide) (PNIPAM) chains was studied by atomic force microscopy based single molecule force spectroscopy. Force-extension curves obtained in water below and above the lower critical solution temperature, in the co-nonsolvent water/methanol mixture, and in dimethyl sulfoxide (H-bond blocking) follow the same trajectory, independently whether the chain was pulled from a collapsed or from a solvated state. This result indicates that for a single PNIPAM chain the formation of intrachain H-bonds in the precipitated state does not cause measurable chain stiffening at the single chain level.

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4.1 Introduction

Stimulus responsive polymers hold the promise of becoming essential parts of functional devices and systems. These materials respond to small variations in the state parameters of their environment, or upon impact of external fields, by large changes in their macromolecular conformation, chain stiffness, segmental interactions, etc. One of the most-studied stimulus responsive synthetic polymers to date is poly(*N*-isopropylacrylamide) (PNIPAM).¹ PNIPAM exhibits a lower critical solution temperature (LCST) in aqueous media around 32 °C.² At temperatures $T < T_{LCST}$, the polymer is soluble in water, due to the dominating, solvating water-polymer segment interactions, while at $T > T_{LCST}$ the polymer precipitates. This temperature responsive behavior is discussed in numerous studies on the basis of observations at the molecular ensemble level.¹ Here we describe single molecule stretching behavior of PNIPAM by variable-temperature atomic force microscopy based single molecule force spectroscopy (AFM-SMFS) in water and in water-methanol mixtures. AFM-SMFS allows one to study single chain behavior of stimulus responsive macromolecules, including studies of optically responsive systems,^{3,4} organometallic polymers with electrochemical responsive behavior,⁵⁻⁷ as well as pH- and temperature-sensitive chains.

Essential for the phase behavior of PNIPAM is the balance among the various interaction energy terms (segment-segment and segment-solvent), including specific (H-bonding) and nonspecific (van der Waals) contributions, in the solvent. Increasing the temperature from below the T_{LCST} , this balance is significantly perturbed at the transition point, and a coil-to-globule transformation takes place.²

A similar effect can be observed when methanol is added to the polymer-water solution.⁸ Despite the fact that methanol and water are both good solvents for the polymer their mixture becomes a non solvent (co-nonsolvent) in the range of 0.2-0.65 w/w% of methanol, and the polymer precipitates.

4.1.1 The molecular origin of the LCST behavior of PNIPAM

According to Tanaka⁹ *et al.* the random coil structure of the polymer stands for collapsed and hydrated segments even in a soluble state. In their work they propose that a dynamic change takes place around T_{LCST} by transition from collapsed pearls towards fully hydrated chains. Upon increasing the temperature to above T_{LCST} the polymer chain dehydrates, and the amount of the collapsed segments increases.

The polymer segments are hydrated and water molecules are held in place via H-bonds with the amide side groups (C=O— — H—O—H) (Figure 4.1). Inside the 'globules' intermolecular H-bonds (C=O— — H—N) are formed between the amide side groups via water bridges.

In solution, the phase transition is accompanied by the rearrangement of the H-bonded structure. The main driving force for the collapse of the polymer on one hand is the energetically favourable H-bond formation between water-water molecules,

Figure 4.1: The structure of PNIPAM, surrounded with bridging water molecules.

while on the other hand, the interaction between the hydrophobic side chains.¹⁰ In other words, during the transition, the polymer segments lose interaction with water molecules and form intramolecular H-bonds.^{11–13} Furthermore, the CH_3 and CH_2 segments become dehydrated. When approximately a third of the amide bonds become dehydrated, the polymer precipitates.

Co-nonsolvency of PNIPAM in water-methanol solvent mixture¹⁴ occurs due to competitive H-bonding between polymer side chains and solvent molecules.^{9,15,16} H-bonds form between the solvent molecules, notwithstanding the choice of the solvent (water or methanol),¹⁷ consequently increasing the amount of intramolecular H-bonds, thus the polymer precipitates.

The collapse of PNIPAM chains in solution is accompanied by polymer precipitation resulting in turbidity.^{18,19} If PNIPAM brushes or hydrogels are considered, the properties including brush/hydrogel stiffness and surface characteristics also change^{20,21} upon passing the T_{LCST} . Until now, experimental data supporting LCST behavior and its molecular formation have been observed at the polymer ensemble level. In this study we tackle single chain behavior of PNIPAM in solution, from a true molecular perspective, as a function of the temperature or solvent composition.

4.1.2 Single chain force spectroscopy

AFM-SMFS tools allow one to follow, in real time, the movements, forces and strains developed during conformational changes induced by external force.^{22,23} This technique can be used for the detection and mechanical characterization of single molecules, with the advantages of high spatial sensitivity and low signal to noise,

under environmentally controlled conditions.^{24,25}

The polymer should bridge a substrate and an AFM tip, being (covalently or physically) bound to either (or both) of them. The facing surfaces are separated from each other thereby pulling the polymer chains. Here we chose a chemical grafting approach and end-functionalized PNIPAM chains, attached covalently to the AFM probes. Thus substrate-induced conformational changes could be prevented. From

Figure 4.2: Schematic representation of the AFM-SMFS experiment. The enlarged image is a diagram illustrating the 'pearl like' structure of the polymer.

the characteristic force–extension curve, one can obtain information about intra- and intermolecular interactions and the mechanical stiffness of the polymer chain.^{26–28}

Several chain statistical models can be applied to obtain quantitative data regarding the behavior of a single chain upon pulling.²⁹ The pulling force to uncoil the polymer chain in the worm like chain (WLC) model^{30,31} is given as a function of the extension, thus it can be applied directly when a force–extension curve is obtained using AFM-SMFS; see equation 4.1:

$$F(z) = \frac{k_b T}{l_p} \left[\frac{1}{4} \left(1 - \frac{z}{L_c} \right)^{-2} + \frac{z}{L_c} - \frac{1}{4} \right] \quad (4.1)$$

where $F(z)$ is the applied force, z is the extension, l_p is the persistence length and L_c is the contour length of the polymer, k_b is the Boltzmann constant and T is the temperature.

The model describes a polymer chain that is intermediate between a rigid-rod and a flexible coil taking into account both local stiffness (described by the persistence length, l_p) and long-range flexibility. The chain is treated as an isotropic, homogeneous rod whose trajectory varies continuously and smoothly through space.

We note that it might be more appropriate to use the modified freely jointed chain model³² over the WLC description. However, in our case fitting with WLC provided good correlation with experiments. Further support for the choice of the WLC is provided by the good overlap of the normalized force–extension curves.³³

Single chain stretching experiments at room temperature on PNIPAM was previously reported by Zhang *et al.*³⁴ These measurements were performed in water and in 8 M urea solution, with differences being found in the elasticity parameter upon changing the environment similar to the pH dependent behavior of other polyacrylamides.³⁵ Additionally, the PNIPAM samples had been heated to 33 °C, kept for 10 min and the original RT experiments were performed again. Unfortunately, numerical fitting, regarding values of chain-statistical parameters, was inconclusive in that study. The authors concluded that during the heat cycle the adsorption of the PNIPAM chains to the surface changes, which was considered a limitation for reproducing the initial force curves (obtained prior to temperature treatment). The question thus remains open whether there is a single chain signature of a thermodynamic first-order phase transition when single chain elasticity is considered.

4.2 Results and Discussion

Figure 4.3 shows the normalized force–extension curves obtained during the AFM-SMFS measurement. The range of desorption force of the fully extended polymer chain after stretching was found to be between 80–700 pN, indicating breaking of physical attachments rather than chemical, covalent bond rupture.³⁶ First the polymer was studied below and above its T_{LCST} (32 °C). Force curves were normalized by the corresponding chain contour length, obtained from the WLC fit, and plotted in 4.3a. At the 3 different temperatures measured, namely 26, 36, an 40 °C, the same trajectory was observed and hence the force curves superimpose well regardless of temperature variation. Normalized force–extension curves obtained in different solvents are plotted in 4.3b. Stretching behavior of the PNIPAM chains in the mixture of H₂O/MeOH and in DMSO show the same trajectory as in water.

In some cases, multiple rupture events as well as desorption plateaus were observed. These force–extension curves were discarded, due to the absence of a clear baseline.

At small tip-surface separations an adhesion peak is observed. This non-specific adhesion is probably caused by high affinity of PNIPAM to gold surfaces³⁷ (hydrophobic interaction) as well as the interaction between the OH terminated monolayer on the tip surface and the underlying substrate. These forces did not show any conclusive trend regarding temperature or solvent, therefore they have not been further analysed.

The obtained force–extension curves were fitted to the WLC model. The values determined for the fitted persistence length (l_p) are summarized in Table 4.1. The obtained values are in each others error range, in other words, there is no significant

Figure 4.3: Superimposed force–extension curves (a) at three different temperatures, 26, 36 and 40 °C; (b) in different solvents, normalized by their L_c , obtained from the WLC fit.

difference in the stiffness of the PNIPAM chains in different environments.

In Figure 4.4, L_c values obtained during a set of measurements —performed below (26 °C) and above (36 °C) the LCST— are presented in a histogram. The length of the observed polymer chain bridges was between 20 and 200 nm. The polymer is covalently attached to the AFM tip at one end, but to the opposite surface it is adsorbed via a segment. From the histograms it can be gathered that the polymer chains are physisorbed to the substrate somewhere in their 'middle' section as opposed to their chain ends.³⁸ The most probable length was found to be 37.9 ± 0.8 nm at 26 °C and 44.7 ± 1.0 nm at 36 °C.

Figure 4.4: Histograms of the contour lengths (L_c) at different temperatures, (a) 26 °C and (b) 36 °C with corresponding Gaussian fit.

Table 4.1: Average persistence length (l_p), obtained from fitting the force curves in different solvents and at different temperatures using the WLC model.

Solvent	T [°C]	l_p [nm]
H ₂ O	26	0.51 ± 0.09
H ₂ O	36	0.45 ± 0.06
H ₂ O	40	0.53 ± 0.26
H ₂ O/MeOH	26	0.54 ± 0.12
DMSO	26	0.49 ± 0.09

The unfolding of the polymer chain follows the same trajectory in all cases measured, increase of temperature or mixed solvents. This is an unexpected observation in view of clear and well established differences in behavior observed in the bulk. Earlier it was shown that the T_{LCST} of the polymer in water can be shifted to higher temperatures, or even suppressed, by increasing mechanical tension on the polymer chains.^{9,39} This phenomenon was explained by forced solvation of the collapsed polymer segments at high pressure. Applying a similar argument here, we could explain the observed behavior of the polymer chain during the AFM-SMFS measurement. In our case, an external force is applied to the bridging chain, pulling it out from the collapsed globule. As the chain starts to uncoil, it becomes gradually exposed to the surrounding solvent molecules. Considering the schematic representation of the polymer chain (see Figure 4.2) in between the opposing surfaces, thus the effective number of the pearl-like collapsed sites decreases upon uncoiling. Since the segments are getting further away from each other during pull, they become easier solvated. Segment hydration is promoted by 'positive correlation' between water molecules bound to the chain⁹; since binding of a water molecule onto a site next to an already bound one is more preferential. Such uncoiling of the polymer chain should eventually result in the same structure as in the soluble state.⁹ Measurements in DMSO also indicate the lack of a strong intrachain H-bond stabilization. DMSO is a well-known solvent for breaking H-bonds,⁴⁰ thus it can break up globules held together by intramolecular H-bonds. The fact of identical stretching trajectories observed in DMSO and for the precipitated polymer at high temperature or in the mixed solvents confirms a dynamic, weakly bounded structure as proposed by Ono et al.⁴¹ Finally, we note that reports indicate disruption of co-operative H-bonded structure for poly(ethylene glycol) for stretching forces below 100 pN.²⁷ In our measurement, desorption forces we observed for low extensions cloud the possibility to observe low force H-bond rupture events, since the chain must be stretched to 20–30% before a meaningful stretching curve can be detected.

4.3 Summary and Conclusion

Stretching behavior of single PNIPAM chains, grafted to Au-coated AFM tips, was monitored in single chain AFM force spectroscopy experiments. No change in the single chain force–extension curves below and above the T_{LCST} and in a mixture of $H_2O/MeOH$ was found. This result indicates that under high mechanical stress no chain stiffening can be observed at the single molecule level. This was further evident from measurements in DMSO as a solvent, by suppressing intrachain H-bonds, where the same trajectory of stretching was found as in the non-soluble state. Chain stiffness may change on the single chain level, but not under the experimental conditions used in this study.

4.4 Experimental section

Chemicals were purchased from Sigma-Aldrich (The Netherlands) and used as received if not stated otherwise. Organic solvents were purchased from Biosolve (Valkenswaard, The Netherlands), hydrogen peroxide (H_2O_2) and ethanol were purchased from Merck (The Netherlands). *N*-isopropylacrylamide (Acros Organics, Belgium) was recrystallized from hexane/toluene. Deionized water from a MilliQ Advantage A 10 purification system (Millipore, Billerica, Ma, USA) was used.

Synthesis of PNIPAM:

α,ω thiol end-functionalized PNIPAM was synthesised by UV initiated RAFT polymerization. *p*-Xylylene bis(*N*, *N'*-diethyldithiocarbamate) (XDC) was used as photoiniferter.⁴² The photoinitiator was synthesised by adding an equivalent amount of *p*-dichloroxylylene to sodiumdithiocharbamate in ethanol, and the resulting yellowish powder was isolated by filtration. The photoiniferter and the NIPAM monomer (100 mg) were charged into a quartz cuvette in a ratio 1:1000 in 1 ml dimethylformamide (DMF). The polymerization was carried out with a $\lambda=365$ nm LED light source⁴³ for 2 hrs, and the obtained product was precipitated into diethylether. To cleave the dithiocarbonate end functions, the polymer was reacted with an excess of hexylamine in DMF overnight, precipitated into diethylether followed by pentane and then dried in vacuum. The polymer was characterized by gel permeation chromatography (GPC). 0.1 M LiCl in DMF was used as an eluent⁴⁴ against poly(methyl metacrylate) standards. ($M_w = 582$ kDa, PD = 2.2).

AFM tip functionalization:

For chain attachment to the AFM probes, Au-thiol chemistry was used.⁴⁵ A solution was prepared with the thiol end-functionalized polymer (1 mg/ml) and 11-mercapto-1-undecanol (10^{-6} M) in methanol. Gold coated Bio-lever (Asylum Research, Santa Barbara) or V-shaped contact mode probes (Bruker, Santa Barbara) were placed in the solution for overnight to achieve PNIPAM grafting.

AFM measurements:

A Bruker Multimode AFM with a heater-cooler setup and a NanoScope V controller was used for the variable temperature measurements and a PicoForce setup with NanoScope IVa controller was employed in other cases, both equipped with a closed quartz liquid cell as a tipholder. The modified tip was mounted into the liquid cell and as a surface H_2 annealed (111) gold substrates (Arrandee, Germany) were used, which were previously cleaned with piranha solution (3:7 V/V% H_2O_2 : H_2SO_4) and washed with water and ethanol. The force curves were captured in water, dimethylsulfoxide or water/methanol ($\text{H}_2\text{O}/\text{MeOH}$) (50 V/V%) mixture, with a fixed retraction rate, 200 nm/s, applying 1 s surface delay. The deflection sensitivity was determined on a silicon substrate, after which the spring constant of the cantilever was measured using the thermal noise calibration procedure.⁴⁶ The measured spring constants were 0.0059 ± 0.003 N/m for the Biolevers and in the range of 0.08 ± 0.03 N/m for the V-shaped contact mode probes. The force–extension curves were analysed with NanoScope 7 software. The WLC model was fitted using a fast converging non-linear least-square fit based on the Marquardt-Levenberg algorithm.

4.5 References

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Chapter 5

Synthesis of dual stimuli responsive molecular bottlebrushes with a redox responsive poly(ferrocenylsilane) backbone and temperature responsive poly(N-isopropylacrylamide) side chains

Dual stimuli responsive molecular bottlebrushes were obtained using 'grafting to' and 'grafting from' processes. As a backbone redox responsive poly(ferrocenylsilane) (PFS), and as side chains temperature responsive poly(N-isopropylacrylamide) (PNIPAM) were used. The 'grafting to' method involved a Huisgen cycloaddition click reaction between an azide functionalized PFS backbone and alkyne end-functionalized PNIPAM chains with various molar masses. According to ^1H NMR-, ATR-FTIR spectroscopy and GPC measurements near-quantitative azide functionalization of the PFS was achieved and the polymer backbone remained intact during the reaction. Furthermore, the click reaction between the backbone and the side chains afforded bottlebrushes with an average molar mass of $M_n = 2\text{-}4$ million g/mol. In the click reaction, approximately 75% of the side chains were functionalized with PNIPAM, yielding bottlebrushes with relatively high grafting density. In the 'grafting from' method, ATRP initiator-, RAFT transfer agent - or photoiniferter moieties with alkyne end-functionality were connected as side groups to the azide functionalized PFS backbone. In addition, an ATRP initiator moiety was attached to amine functionalized PFS to form an organometallic macroinitiator. Near-quantitative side group functionalization was achieved in each case. The polymerization reaction environment had to be carefully tuned because in some cases due to steric hindrance during the 'grafting from' process the backbone was damaged. The bottlebrush obtained via an ARGET-ATRP process had a molar mass $M_n = 0.8$ million g/mol.

5.1 Introduction

Molecular bottlebrushes, belonging to the class of graft (co)polymers, are macromolecular architectures, composed of a linear backbone with densely grafted side chains. Due to steric hindrance between the side chains, bottlebrushes adopt an extended, cylindrical conformation.^{1–3} The construction of bottlebrushes from stimuli responsive polymer chains opens up new application opportunities in various fields. Reversible responses of the macromolecular architectures, triggered by variations in pH,^{4,5} temperature,^{6,7} magnetic field strength or by irradiation with light, enable their use in areas such as actuation,⁸ controlled drug delivery^{9,10} and many others.¹¹ Furthermore, the development of bottlebrushes responsive to multiple stimuli is expected to yield novel, fascinating addressable materials.³

Varying the chemical composition allows one to easily tune the nature and rate of the response, which is greatly aided by the developments in controlled radical polymerization methods. Molecular bottlebrushes can be prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization,^{12,13} atom transfer radical polymerization (ATRP),^{14,15} nitroxide mediated polymerization (NMP),¹⁶ ionic polymerization¹⁷ and ring-opening metathesis polymerization.¹⁸

Synthesis of defined molecular bottlebrushes requires the growth of polymer chains with a controlled degree of polymerization. A polymer backbone with reactive pendant groups that can initiate chain growth is required for bottlebrush construction using the 'grafting from' method. Alternatively, bottlebrushes are obtained from backbones possessing pendant moieties that allow attachment of pre-formed, end-reactive chains in a 'grafting to' process.^{19,20} A third method employs polymer chains with a polymerizable end group which can form molecular bottlebrushes in a 'grafting through' process. This method, however, often leads to relatively short backbones due to the low concentration of the polymerizable groups and steric hindrance of the side chains.

Although grafting densities achieved by employing the 'grafting to' method are limited for steric reasons, the method remains attractive as side chains of defined degrees of polymerization can be prepared and characterized thoroughly before their attachment to the bottlebrush main chain. The development of efficient coupling methods such as the Cu(I) catalyzed Huisgen cycloaddition between an azide and an alkyne group,²¹ has helped to increase grafting densities in 'grafting to' processes and therefore has increased the viability of this approach. The Huisgen cycloaddition has proved to be a versatile, highly reliable coupling reaction that proceeds with high yields under mild conditions, has a high tolerance for other functional groups, and has demonstrated its applicability for polymer modification in solution.^{21–25}

Here, we describe the synthesis of dual responsive bottlebrushes composed of a redox responsive poly(ferrocenylsilane) (PFS) main chain and temperature responsive poly(*N*-isopropylacrylamide) (PNIPAM) side chains. Interestingly, the majority of stimuli responsive bottlebrushes are obtained in ATRP 'grafting from' processes from poly(2-bromoisobutryloxyethyl methacrylate) (PBIEM) backbones, therefore

displaying only side chain stimuli responsiveness.

Figure 5.1: General structure of the polymers.

Properties of redox responsive PFSs (Figure 5.1) are influenced to a large degree by their side groups. Attachment of water soluble blocks^{26,27} or ionic side groups for instance allows one to dissolve the hydrophobic PFS backbone in water.²⁸ Usually, substituents are introduced at the silicon atom, which may for instance possess a reactive chloro atom, halo-alkyl group, double bond²⁹ or anionic/cationic side groups, offering a site for further modification.^{30,31}

PNIPAM is one of the most often used polymers for the preparation of temperature responsive bottlebrushes,³ since it can be obtained by controlled radical methods such as RAFT³² or ATRP.^{33–35} In aqueous solution, PNIPAM exhibits a lower critical solution temperature (LCST) around 32 °C.³⁶ At lower temperatures the polymer is soluble in water, while above the LCST the polymer precipitates.

Figure 5.2: Schematic presentation of 'grafting to' and 'grafting from' processes based on the Huisgen cycloaddition reaction and the formed connecting group, a triazole ring.

To obtain a dual stimuli responsive macromolecule, a 'grafting to' and a 'grafting from' process are used. The 'grafting to' method involves a Huisgen cycloaddition click reaction between an azide functionalized PFS backbone and alkyne end-functionalized PNIPAM chains.²² In the 'grafting from' method, ATRP initiator moieties were connected as side groups to the PFS backbone to form an organometallic macroinitiator. Figure 5.2 summarizes these approaches. This Chapter discusses the various routes to PFS-PNIPAM molecular bottlebrushes that were explored.

5.2 Results and Discussion

5.2.1 Azide functionalized backbone

To obtain a molecular bottlebrush, a PFS backbone was functionalized with pendant azide groups to be applied in the Azide-alkyne Huisgen cycloaddition (click reaction). A generic method for obtaining PFS chains with pendant functional groups involves the ring-opening polymerization of [1](3-chloropropyl)methylsilaferrocenophane to poly(ferrocenyl(3-chloropropyl)methylsilane) (**PFS-Cl**), which can be derivatized further by nucleophilic substitution reactions.³¹ For instance, it can be converted into its more reactive iodo analogue, poly(ferrocenyl(3-iodopropyl)methylsilane) (**PFS-I**), allowing a wider range of functional moieties to be attached in subsequent side group conversion reactions. To obtain PFS chains with azide side groups, either **PFS-Cl** or **PFS-I** was reacted with NaN_3 , yielding poly(ferrocenyl(3-azidopropyl)methylsilane) (**PFS-N₃**).³⁷

Scheme 5.1: a) Azide functionalization of PFS and b) the corresponding ¹H NMR spectra.

¹H NMR spectroscopy shows that the peak belonging to 3-CH₂, directly adjacent to Cl, I or N₃, (Figure 5.1b), shifts to a new position following each derivatization. Clearly, near-quantitative side group conversion was achieved in each reaction. This was further evidenced by ¹³C NMR spectroscopy, showing the expected chemical shifts and a complete disappearance of starting material peaks. Complete substitution in case of **PFS-I** was achieved at room temperature in tetrahydrofuran (THF), while **PFS-Cl** required the presence of a polar aprotic solvent (DMSO) and moderate heating to fully convert it into **PFS-N₃**. For defined molecular bottlebrushes, side group interconversions must proceed quantitatively and the backbone must remain

intact during these steps. To ensure the absence of chain scission, gel permeation chromatography (GPC) measurements were performed (Figure 5.3). The shapes of the peaks are similar, but the **PFS-N₃** peak has shifted to a higher elution volume with respect to the **PFS-Cl** signal, suggesting a lower molar mass for **PFS-N₃**. Polystyrene-effective molar masses and polydispersities for these polymers are summarized in Table 5.1.

Table 5.1: Molar mass characteristics of bottlebrush building blocks for 'grafting to' assembly.

Polymer	$M_{n,NMR}^a$ 10 ³ g/mol	$M_{n,GPC}$ 10 ³ g/mol	$M_{w,GPC}$ 10 ³ g/mol	PDI	$DP_{n,GPC}$	$DP_{n,NMR}$
PFS1	n.a	169.2 ^{b,e}	372.8	2.20	429	
PFS-N₃1	n.a	112.3 ^{b,e}	227.4	2.02	n.a	
PFS2	n.a	94.2 ^{b,d}	284.2	3.02	303	
PFS-N₃2	n.a	45.8 ^{b,d}	346.8	7.60	n.a	
PFS3	n.a	302.0 ^{b,d}	594.6	1.97	965	
PFS-N₃3	n.a	61.3 ^{b,d}	294.4	4.80	n.a	
PNIPAM1	10.5	11.7 ^c	12.8	1.09	104	93
PNIPAM2	19.0	26.3 ^c	30.2	1.15	233	168
PNIPAM3	6.3	6.3 ^b	7.3	1.16	56	56

^aObtained from ¹H NMR, based on alkyne-CH₂ and PNIPAM repeat unit integral values.

^bMeasured in THF against narrow polystyrene standards. ^cMeasured in 0.1 M LiCl/DMF against well-defined poly(methyl methacrylate) standards. ^dPFS-Cl, ^ePFS-I.

Figure 5.3: GPC traces of (a) **PFS-Cl** (black) and its derivative **PFS-N₃** (grey) and (b) **PFS-I** (black) and its derivative **PFS-N₃** (grey).

However, as the polydispersities are similar and no obvious signals belonging to

low molar mass polymer chains are present, chain scission can be ruled out. The lower polystyrene–effective molar mass of **PFS-N₃**, compared to the **PFS-Cl** is most likely caused by a smaller hydrodynamic volume of the **PFS-N₃** coil, due to dipolar intramolecular interactions between its azide moieties.

5.2.2 Synthesis of molecular bottlebrushes via a 'grafting to' method

The bottlebrush backbone, functionalized with an azido moiety at each repeat unit, is now available to attach PNIPAM chains, end-functionalized with an alkyne group. Well-defined PNIPAM chains with desired end groups are accessible by the RAFT process or by ATRP processes. To investigate the influence of side chain length on the properties of the bottlebrushes, alkyne end-functionalized PNIPAM of varying molar mass was synthesized. The alkyne group was introduced via an alkyne-bearing chain-transfer agent for RAFT, (S-1-dodecyl-S'-(α,α' -dimethyl- α'' -propargyl acetate) (**DDAT**) or an alkyne-functional ATRP initiator (3-(trimethylsilyl)prop-2-yn-1-yl 2-chloropropanoate (**Si-PCP**)). The RAFT process was conducted according to a literature procedure where the CTA concentration was adjusted to control the molar mass.³⁸ PNIPAM chains of $M_n = 6.3 \times 10^3$ or 26.3×10^3 g/mol were obtained as established by GPC and end-group analysis using ¹H NMR spectroscopy (**PNIPAM2** and **PNIPAM3** Table 5.1).

To obtain PNIPAM via ATRP proved to be quite challenging. In order to protect the alkyne group during the polymerization from complexation with the Cu catalyst employed with ATRP, it was capped with a trimethylsilyl group.³⁹ The polymerization typically resulted in a low yield of product. The highest achieved monomer conversion was around 50%, obtained by polymerization in a DMF/iPrOH mixture and leading to a PNIPAM molar mass $M_n = 11.7 \times 10^3$ g/mol **PNIPAM1**. We note that the low yields might be due to catalyst complexation by NIPAM and PNIPAM. After polymerization, the trimethylsilyl protecting group was removed from the PNIPAM chains ends using tetrabutylammonium fluoride.

Table 5.2: Molar mass characteristics of bottlebrush polymers by the 'grafting to' method.

Name	$M_{n,GPC}^a$ x10 ⁶ g/mol	$M_{w,GPC}$ 10 ⁶ g/mol	PDI	PNIPAM $DP_{n,NMR}^b$	PNIPAM $M_{n,NMR}^b$ 10 ³ g/mol	$M_{n,calc}$ 10 ⁶ g/mol
Bb1	2.143	2.519	1.18	38	4.3	1.986
Bb2				133	15.0	4.613
Bb3				42	4.7	4.835

^aMeasured in THF against narrow polystyrene standards. ^bObtained from ¹H NMR, based on PFS (Si-CH₃) and PNIPAM repeat unit integral values.

The click reaction which attaches the PNIPAM chains to the PFS backbone was conducted in THF in the presence of a Cu^0/Cu^I catalyst system, that was formed in situ by reduction of Cu^{II} to Cu^0 with Na-ascorbate.²⁴ To remove the reduced Cu, the solution was centrifuged. Subsequently, unreacted PNIPAM chains were removed by dialysis, using large-pore dialysis tubing. The solubility of the dark yellow–amber product in water already gave an indication of the successful PNIPAM attachment to the hydrophobic PFS backbone. Interestingly, when 5% DMSO was added to the reaction mixture, the click reaction proceeded faster. **Bb1**, **Bb2** and **Bb3** were obtained by clicking **PNIPAM1** to **PFS-N₃1**, **PNIPAM2** to **PFS-N₃2** and **PNIPAM3** to **PFS-N₃3**, respectively.

First, the molecular bottlebrushes were characterized by means of ATR-FTIR spectroscopy. For all bottlebrushes, ATR-FTIR spectra (Figure 5.4) clearly show, besides the characteristic peaks of PNIPAM at 1635 and 1535 cm^{-1} of the amide group, a characteristic peak at 1036 cm^{-1} , belonging to the out-of-plane vibration of the ferrocene rings.⁴⁰ Compared to other PFS-PNIPAM composite structures in the literature⁴¹ possessing a few wt% PFS in PNIPAM, relative signal intensities of the ferrocene out-of-plane vibration and the PNIPAM carbonyl stretch at 1635 cm^{-1} in the FTIR spectra suggest that the bottlebrushes contain around 1–5% PFS. The absence of the azide stretching peak at 2088 cm^{-1} in all bottlebrush spectra provides evidence for completion of the click reaction. Considering that this absorption appears as a strong signal in the spectrum of the **PFS-N₃** polymer, one may conclude that the efficiency of the grafting reaction and therefore the obtained grafting densities are high.

The bottlebrush molecules were characterized further by means of GPC measurements. As mentioned, the bottlebrush polymers were purified by dialysis which removed the excess of PNIPAM chains. Figure 5.5 displays the GPC traces of the PFS backbone with pendant azide groups (**PFS-N₃1**), the alkyne end-functionalized PNIPAM grafts obtained by ATRP (**PNIPAM1**), and the bottlebrush polymer resulting from the click coupling of these building blocks (**Bb1**), measured in THF. A new peak, appearing in the GPC trace at lower elution volumes, is attributed to the high molar mass bottlebrush polymer. The relatively narrow molar mass distribution derived from this signal may not be representative for the polydispersity of this bottlebrush since their molar mass ($M_w = 2.5 \times 10^6$, $M_n = 2.1 \times 10^6$ g/mol, $M_w/M_n = 1.2$) is near the upper limit of the effective fractionation range of the GPC columns. Nevertheless, the peak corresponding to the PFS backbone at intermediate elution volumes is no longer present in the bottlebrush trace. In some cases, small amounts of unattached, linear PNIPAM chains were still present in the GPC traces of the bottlebrushes. The molar mass characteristics of the various bottlebrush polymers are summarized in Table 5.2. Since the effective fractionation range of our GPC columns extends up to around 2 million g/mol, bottlebrushes with higher estimated molar masses were not measured with GPC.

Even though there is a surplus of PNIPAM repeat units compared to the PFS repeat units, signals of both are present in the ^1H NMR spectra of the bottlebrushes.

(a)

(b)

Figure 5.4: (a) ATR-FTIR spectra of the molecular bottlebrushes. As a comparison, spectra of **PFS- N_3 2** and the three PNIPAM side chains are included. The completion of the click reaction is apparent from the absence of the azide stretching peak at 2088 cm^{-1} . (b) Magnification of (a) between 1300 and 900 cm^{-1} , each bottlebrush has a peak at 1036 cm^{-1} belonging to the out-of-plane vibration of the ferrocene rings.

Figure 5.5: GPC traces of **Bb1** (black), **PFS-N₃1** backbone (grey) and **PNIPAM1** (light grey). The trace of the molecular bottlebrush does not show any peak at the retention volume of the backbone, although un reacted side chains are still present.

On the basis of M_n values of the backbone and the side chains, one may estimate the molar mass of the bottlebrush molecules. For example, **Bb1** has a PFS main chain with a degree of polymerization $DP_n = 423$, as established by GPC measurements. **PNIPAM1** chains with a molar mass $M_n = 4\,300$ g/mol, established by ^1H NMR based on the trimethylsilyl group of the employed ATRP initiator, were attached. If grafting occurred at each repeat unit, the total molar mass of the bottlebrush becomes $M_{Bb1} = 169\,000 + 423 \times 4\,300 = 1.986 \times 10^6$ g/mol. Similarly, for **Bb2** and using the signals from the RAFT initiator for calculating the **PNIPAM2** M_n , a $M_{Bb2} = 94\,000 + 303 \times 15\,000 = 4.613 \times 10^6$ g/mol is found. For **Bb3** M_n , a $M_{Bb3} = 300\,000 + 965 \times 4\,700 = 4.835 \times 10^6$ g/mol is calculated.

In principle, one might estimate grafting density by comparing actual ^1H NMR integral ratios of the PFS and PNIPAM repeat units with ratios calculated for fully grafted bottlebrushes. Due to polydispersity effects and the limited accuracy of the NMR integrals of the weak PFS signals, however, this may lead to less reliable values. Still, one can compare measured ^1H NMR integral ratios of PNIPAM and PFS repeat units with ratios estimated for completely grafted backbones as discussed above. The theoretical and estimated molar masses resulting from this are given in [Table 5.2](#).

Thermogravimetric analysis of the obtained bottlebrush molecules supports the result of the previously mentioned measurements ([Figure 5.6](#)). The decomposition patterns of **PFS-N₃2** and **PNIPAMs** are clearly different. **PNIPAMs** were stable up to 350–400 °C depending on the molar mass of the polymer, and left a low residual weight. **PNIPAM3** displayed two decomposition steps. At 250 °C the polymer lost 5% weight and decomposed at 380 °C, leaving a residual weight of 1.4%.

Figure 5.6: Thermogravimetric analysis of the bottlebrush molecules. As a reference PFS- N_3 and the three PNIPAM side chains are shown. The inset presents a magnification of the region between 400 and 600 °C.

PNIPAM1 and **PNIPAM3** were stable up to 380 °C and after decomposition, 1.0 and 0.6 wt.% remained, respectively. The bottlebrush molecules followed a similar decomposition path as PNIPAM, but resulting in a higher residual weight. **Bb2** decomposed in two steps, losing 5% weight at 150 °C, and at 345 °C finally resulting in the lowest residual weight (2.8 wt.%). Indeed, **Bb2** had the largest amount of PNIPAM. **Bb3** decomposed at 345 °C resulting in 5% residual weight, while the less PNIPAM containing bottlebrush **Bb1** left 5.9 % weight upon decomposition at 345 °C. Decomposition of **PFS- N_3 2** occurred at 250 °C and the 43% residual weight originates from the remaining iron and silicon, which are responsible for the residual weight of the bottlebrushes as well.

5.2.3 Synthesis of molecular bottlebrushes via a 'grafting from' method

Synthesis of ATRP, RAFT and photoiniferter macroinitiators

In addition to forming cylindrical molecular bottlebrushes by a 'grafting to' process, a 'grafting from' method was used to grow PNIPAM side chains from a PFS macroinitiator. Three different types of initiator for controlled radical polymerization were attached to a PFS backbone: (i) an ATRP initiator, 3-(trimethylsilyl)prop-2-yn-1-yl 2-chloropropanoate (**PCP**),^{34,39} (ii) a RAFT initiator, S-1-dodecyl-S'-(α,α' -dimethyl- α'' -propargyl acetate) (**DDAT**),³⁸ and (iii) a photoiniferter, 4-ethynylbenzyl diethylcarbamodithioate (**EBDC**).⁴² All three initiators were attached to an azido-

Scheme 5.2: Synthesis of the macroinitiator: **PFS-PCP**, **PFS-DDAT**, **PFS-EBDC**.

functional PFS backbone by 'click' chemistry, as described previously for the 'grafting to' attachment of PNIPAM chains (Scheme 5.2). Furthermore, an ATRP initiator was attached to an amine-functional PFS main chain by treating this backbone with 2-chloropropionyl chloride in the presence of triethylamine. The initiator molecules, possessing an alkyne functionality, were clicked to **PFS-N₃** backbones via Cu⁰/Cu^I catalyzed cycloaddition reaction, yielding the polymers **PFS-PCP**, **PFS-DDAT** and **PFS-EBDC**.

The Cu⁰/Cu^I was introduced via reduction of copper salts such as CuCl or CuSO₄ with Na-ascorbate. We observed that CuCl is not suitable as reactant here, as it seemed to cleave the PFS backbone, probably due to attack by the chloride anion. Similar detrimental effects were also seen when CuCl was used as the catalyst precursor in ATRP (for 'grafting from' by ATRP, see in the next section). The best option was found to be CuSO₄, reduced in situ in the reaction medium with Na-ascorbate, Cu⁰/Cu^I. Interestingly, chloride anions are known to degrade ferrocene derivatives during electrochemical measurements. Anionic species inert to ferrocenes include perchlorate and sulfate anions.

The various macroinitiators were characterized by means of ¹H NMR spectroscopy

Figure 5.7: (a) ^1H NMR spectra of the macroinitiators. For guidance for symbols see [Scheme 5.2](#). The completion of the click reaction can be followed by the shift of the peaks: **L** shifts from $\delta = 0.9$ to 0.8 ppm, **K** from $\delta = 1.6$ to 1.8 ppm while **J** shifts from $\delta = 3.2$ to 4.2 ppm. The signal **F** of the triazole ring appears at 7.4 ppm. (b) ATR-FTIR spectra of the macroinitiators. The completion of the click reaction is apparent from the absence of the azide stretching peak at 2088 cm^{-1} in the trace of the macroinitiators.

(Figure 5.7a). The formation of the triazole ring was evident from the appearance of a peak at 7.4 ppm, belonging to **F**. The signals of the methylene units of the propyl side chain shift as the side groups are interconverted: **L** shifts from $\delta = 0.9$ to 0.8 ppm, **K** from $\delta = 1.6$ to 1.8 ppm while **J** shifts from $\delta = 3.2$ to 4.2 ppm as the azido group is turned into a triazole ring. Besides these changes, the proton signals from the clicked functional groups appear in the spectra as well. In addition, ^{13}C measurements were performed, confirming the full conversion of the azido group. The high efficiency of the 'click' reaction was further supported by FTIR spectroscopy. For all macroinitiators, the absence of the absorbance at 2088 cm^{-1} , characteristic of azide groups and well-separated from other clearly resolved vibrational and stretching absorption peaks, confirms the full conversion of the azide groups to triazole rings (Figure 5.7b).

Synthesis of the macroinitiator from amine-functional PFS

The previous route to PFS macroinitiators was based on a click chemistry approach. Other options for attaching ATRP initiator moieties to PFS chains were also explored, including amide bond formation reactions. **PFS-Cl** was converted into poly(ferrocenyl(3-aminopropyl)methylsilane) **PFS-NH₂** according to the procedure reported in the literature.^{28,43} **PFS-NH₂** was treated with 2-chloropropionyl chloride to introduce pendant ATRP initiator groups, resulting in the macroinitiator **PFS-N-PCP** (Scheme 5.3).

Scheme 5.3: Synthesis of **PFS-N-PCP**: reaction scheme of the coupling of the ATRP moiety to amine functionalized PFS.

The macroinitiator was characterized by means of ^1H NMR spectroscopy (Figure 5.8a). The formation of the amide bond was evident from the appearance of a peak at 6.6 ppm, belonging to the N-H group **C**. Furthermore, characteristic peaks at

Figure 5.8: (a) ^1H NMR spectrum of **PFS-N-PCP**. The completion of the reaction can be followed by the shift of the peaks: **L** shifts from $\delta = 1$ to 0.9 ppm, **K** from $\delta = 1.8$ to 1.6 ppm while **J** shifts from $\delta = 3.5$ to 3.2 ppm, **B** of the initiator moiety appears at $\delta = 4.4$ and **C** at $\delta = 6.6$ ppm of the initiator moiety. (b) ATR-FTIR spectrum of **PFS-N-PCP**, the appearance of the characteristic amide peaks indicates the completion of the reaction: Amide I at 3290 , 1655 and Amide II at 1528 cm^{-1} .

$\delta = 1.8$ ppm belonging to **A** and $\delta = 4.4$ ppm belonging to **B** appear in the spectrum. The signals of the methylene units of the propyl side chain shift as well, as the side

groups are interconverted: **L** shifts from $\delta = 1$ to 0.9 ppm, **K** from $\delta = 1.8$ to 1.6 ppm while **J** shifts from $\delta = 3.5$ to 3.2 ppm as its neighboring amino group is turned into an amide group. In addition, ^{13}C NMR measurements were performed, confirming the full conversion of the chloro group. The completion of the reaction was further supported by FTIR spectroscopy by the appearance of the characteristic amide peaks. The new absorptions, Amide I, N-H symmetrical-asymmetrical stretching vibration at 3290 cm^{-1} , the C=O stretching vibration at 1655 and amide II at 1528 cm^{-1} , are present in the spectrum and well-separated from other clearly resolved vibrational and stretching absorption peaks (Figure 5.8b).

Table 5.3: Molar mass characteristics of the macroinitiator.

Macro-initiator	Precursor	$M_{n, GPC}$ 10^3 g/mol	$M_{w, GPC}$ 10^3 g/mol	PDI
PFS-N-PCP	PFS2	41.2 ^a	90.6	2.20

^aMeasured in THF against narrow PDI polystyrene standards.

5.2.4 Grafting PNIPAM from PFS macroinitiator

As described in Section 2.3, four macroinitiators were obtained, two of them possessing pendant ATRP initiator moieties (**PFS-N-PCP** and **PFS-PCP**), one with a RAFT chain transfer agent (**PFS-DDAT**) and one with a photoiniferter pendant group (**PFS-EBDC**). Several criteria must be fulfilled for a successful 'grafting from' process: the macroinitiator and the monomer must be soluble in the polymerization medium, equilibrium between active and dormant species must be established during polymerization, and due to the sensitivity of the PFS backbone, mild polymerization conditions are preferred.

DMF is a commonly used solvent in radical polymerization processes. However, the solubility of the various PFS macroinitiators in pure DMF was not high, therefore, for ATRP and the photoiniferter-mediated polymerization, a THF/DMF mixture was chosen as polymerization medium.

To graft PNIPAM by ATRP, a Cu-catalysed ARGET-ATRP process was applied.^{44–46} In the ARGET procedure for initiating an ATRP, reducing agents are present which constantly regenerate the ATRP activator, the Cu^{I} species, from Cu^{II} species irreversibly formed during termination processes, without directly or indirectly producing initiating species that generate new chains. The amount of Cu-based catalysts in ATRP can therefore be reduced to a few ppm in the presence of appropriate reducing agents. In our case, during polymerization, Cu^{II} was reduced back to Cu^{I} in-situ with ascorbic acid. The employed reaction conditions are similar to the mild conditions used previously for the click reactions, therefore we expect the PFS

Table 5.4: Molar mass characteristics of bottlebrush polymers by the 'grafting from' method.

Name	Macro-initiator	$M_{n,GPC}$ 10 ³ g/mol	$M_{w,GPC}$ 10 ³ g/mol	PDI	$DP_{n,NMR}^a$ 10 ³ g/mol	$M_{n,NMR}^a$ 10 ³ g/mol	$M_{n,calc}$
Bb4	PFS-N-PCP	2 395 ^b	2 882	1.20	22	2.4	869
Bb5	PFS-PCP	4.6 ^b	9.5	2.06			
Bb6	PFS-EBDC	12.9 ^b	24.6	1.90			
Bb7	PFS-DDAT	17.0 ^b	29.3	1.72			
Bb8	PFS-N-PCP	11.6 ^c	20.9	1.80			

^aObtained from ¹H NMR, based on PFS (Si-CH₃) and PNIPAM repeat unit integral values.

^bMeasured in THF against narrow PDI polystyrene standards. ^cMeasured in 0.1 M LiCl/DMF against well-defined poly(methyl methacrylate) standards.

macroinitiator chains to remain intact. RAFT polymerization, using **PFS-DDAT**, was conducted in dioxane, which is a good solvent for the macroinitiator as well as a suitable polymerization medium. Here, the same reaction conditions were used as for the RAFT polymerization of NIPAM, yielding alkyne end-functionalized PNIPAM. The reaction temperature, however, was reduced to 60 °C.

Figure 5.9: GPC traces of **Bb5-Bb7** measured in THF against narrow polystyrene standards. **Bb8** measured in 0.1 M LiCl/DMF against well-defined poly(methyl methacrylate) standards. Each bottlebrush has a low molar mass, $M_{n,GPC} = 4\text{--}17 \times 10^3$ g/mol.

'Grafting from' by photoiniferter-mediated polymerization (Chapter 4) was carried

out using the photoiniferter-functionalized PFS, **PFS-EBDC**, under irradiation of a LED photodiode at $\lambda = 365$ nm. The same irradiation conditions used to prepare PFS-PNIPAM hydrogels, detailed in the literature⁴¹ were employed, thus providing appropriate reaction conditions for PFS.

In all cases, a low monomer concentration was used in the polymerizations to avoid cross-linking of the molecules, and therefore relatively long reaction times ranging from 2-6 hrs were applied. After polymerization, the obtained products were isolated by precipitation into diethyl ether, in certain cases followed by dialysis. Each of the prepared samples showed a good solubility in water.

Figure 5.10: GPC traces of **Bb4** (black) and its macroinitiator: **PFS-N-PCP** (grey).

Table 5.4 summarizes the molar mass characteristics of the obtained bottlebrush molecules. Successful polymerization of NIPAM is obvious from the corresponding ^1H NMR spectra, which show the characteristic signals of PNIPAM, but in some cases PFS proton signals are not visible. GPC traces of the bottlebrushes are plotted in Figure 5.9 and Figure 5.10. The GPC traces of **Bb5-Bb8** show peaks at relatively high elution volumes, indicating products with lower molar masses than expected for bottlebrush polymers (compare with **Bb1**, Figure 5.5). Measured molar masses were between $M_{n, \text{GPC}} = 4\text{--}17 \times 10^3$ g/mol. The polydispersity indices (1.72–2.06) are higher than those of the linear PNIPAMs we obtained under similar reaction conditions and used for click reactions (see Section 5.2.1). Because of the above listed results we suspect that during polymerization chain-scission of the backbone occurred, and the obtained polymers most likely are star-shaped rather than cylindrical bottlebrushes. Rupture of the backbone occurred most probably due to increasing steric hindrance of the growing side chains.⁴⁷

However, under carefully controlled conditions, a bottlebrush molecule was obtained by means of ARGET ATRP. The GPC trace of **Bb4** (Figure 5.10) shows

a signal at a low elution volume, indicative of a high molar mass product. This GPC trace is very similar to the one of the 'grafting to' product **Bb1**, displayed in Figure 5.5.

For **Bb4**, signals of both the PNIPAM repeat units and the PFS repeat units are present in the ^1H NMR spectrum. Based on the M_n values of the backbone, an estimate of side chain length as well as of the molar mass of the bottlebrush molecules can be made. **Bb4** has a PFS main chain with a degree of polymerization $DP_n = 303$, $M_n = 41\,200$ g/mol, $M_w = 90\,600$ g/mol, PDI = 2.2, as established by GPC measurements. To the main chain, PNIPAM chains with $DP_n = 22$ are attached, according to ^1H NMR integral ratios of the PNIPAM characteristic peak (side group C-H) and the PFS-SiCH₃ signal. If grafting occurred at each repeat unit, the total molar mass of the bottlebrush becomes $M_{Bb4} = 94200 + 303 \times 2400 = 869 \times 10^3$ g/mol.

5.3 Summary and Conclusion

Dual stimuli responsive PFS-*g*-PNIPAM molecular bottlebrushes were obtained using 'grafting to' and 'grafting from' processes. The 'grafting to' method involved a click reaction between an azide functionalized PFS backbone and alkyne end-functionalized PNIPAM chains with various molar masses. After quantitative azide functionalization of the PFS main chain, the click reaction between the backbone and the side chains afforded bottlebrushes with an average molar mass of $M_n = 2\text{--}4$ million g/mol. Functionalization of the PFS backbone with pendant initiator groups via click chemistry proceeded with quantitative conversion, and when clicking PNIPAM chains, approximately 75% of the azide groups were engaged, yielding bottlebrushes with relatively high grafting density. Using the 'grafting from' method, PNIPAM chains were grown from the organometallic PFS macroinitiator via an ARGET-ATRP process. RAFT and photoiniferter-mediated 'grafting from' processes require more tuning to become viable.

5.4 Experimental section

Materials

Chemicals were purchased from Sigma-Aldrich (The Netherlands) and used as received if not stated otherwise. Organic solvents were purchased from Biosolve (Valkenswaard, The Netherlands). *N*-isopropylacrylamide (Acros Organics, Belgium) was recrystallized from hexane/toluene. Deuterated chloroform was purchased from Buchem BV (Apeldoorn, The Netherlands). CuCl and CuBr were taken up in 98% acetic acid, stirred overnight, filtered, washed with methanol and dried under vacuum. Deionized water from a MilliQ Advantage A 10 purification system (Millipore, Billerica, Ma, USA) was used. For dialysis, Spectra/Por membrane with $M_w = 1000$ or $15\,000$ g/mol cut off were used. Tris((dimethylamino)ethyl)amine (Me₆TREN) was purchased from Alfa Aesar (USA). Basic

aluminium oxide 60 was purchased from Merck KGaA (Darmstadt, Germany). Vitamin-C was received from Fluka.

Methods

Nuclear Magnetic Resonance (NMR) spectroscopy

^1H NMR spectra were recorded on a Varian Unity Inova (300 MHz) or on a Bruker Avance (400 MHz) instrument. ^{13}C NMR was carried out at 75.5 MHz, on a Varian Unity Inova (300 MHz) instrument. As a reference, the chemical shift of the solvent signal (CDCl_3) $\delta = 7.26$ ppm was used.

Fourier transform infrared (FTIR) spectroscopy

Single reflection attenuated total reflection (ATR) mode FTIR spectra (spectral resolution 4 cm^{-1} , 32 scans) were recorded with a Bruker model Alpha FTIR spectrometer equipped with an ATR platinum diamond 1 reflection crystal (Bruker Optik GmbH, Ettlingen, Germany). Before measurements a background spectrum was recorded against air.

Gel Permeation Chromatography (GPC)

GPC measurements on the polymers were carried out in THF (flow rate 2.0 ml/min) at 25°C , using microstyragel columns (bead size $10\text{ }\mu\text{m}$) with pore sizes of 10^5 , 10^4 , 10^3 , and $500\text{ }\text{\AA}$ (Waters) and in 0.1 LiCl/DMF (flow rate 1.0 ml/min) at 25°C , using microstyragel columns (bead size $10\text{ }\mu\text{m}$) with pore sizes of 10^5 , 10^4 , 10^3 , and $10^6\text{ }\text{\AA}$ (Waters), with a differential refractometer (Waters model 410) detection system. Molar masses were determined relative to polystyrene standards in THF and relative to poly(methyl methacrylate) standards in DMF.

Thermogravimetric analysis

Isothermal weight loss of the polymer samples (5–10 mg) was measured under N_2 environment (flow rate 20 ml/min) as a function of time for different temperatures with a Perkin-Elmer thermogravimetric analyzer (Pyris 1, Waltham, Ma, USA). The temperature was stepped between $50\text{--}600^\circ$ with a rate of $10^\circ/\text{min}$.

Synthesis of (PFS- N_3)

Either poly(ferrocenyl(3-chloropropyl)methylsilane) or poly(ferrocenyl(3-iodopropyl)methylsilane) was used as a starting material to obtain poly(ferrocenyl(3-azidopropyl)methylsilane) **PFS- N_3** . Poly(ferrocenyl(3-iodopropyl)methylsilane) (530 mg, 1.34 mmol), NaN_3 (260 mg, 4 mmol) and 15-crown-5 (800 mg, 3.6 mmol, 0.7 ml) were dissolved in 12 ml THF and stirred for 16 days under N_2 atmosphere, to obtain poly(ferrocenyl(3-azidopropyl)methylsilane) **PFS- N_3** . The polymer was precipitated 2 times into MeOH. Poly(ferrocenyl(3-chloropropyl)methylsilane) (300 mg, 1 mmol), NaN_3 (320 mg, 5 mmol) 15-crown-5 (460 mg, 2.06 mmol, 0.4 ml), were dissolved in the mixture of 10 ml THF and 2 ml DMSO, heated to 50°C and stirred for 16 days under N_2 atmosphere, yielding poly(ferrocenyl(3-azidopropyl)methylsilane) **PFS- N_3** . The product was precipitated 2 times into MeOH and dried in vacuum.

¹H NMR (300 MHz, CDCl₃) of PFS-N₃ 0.45 ppm, (s, 3H, (CH₃-Si), 0.9 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 1.7 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 3.25 ppm (s, 2H, Si-CH₂-CH₂-CH₂-), 3.9 and 4.15 ppm (m, 4-4 H, Cp)

¹³C NMR (75 MHz, CDCl₃) of PFS-N₃ -3.4 ppm (CH₃-Si), 13.4 ppm (Si-CH₂-CH₂-), 23.9 ppm (Si-CH₂-CH₂-), 54.2 ppm (-CH₂-CH₂-N), 69.8 ppm (Si-Cp) 71.3 and 73.2 ppm (Cp)

ATR-FTIR of PFS-N₃, wavenumbers (cm⁻¹) 3086 (C-H stretching vibration for ferrocene), 2931 and 2870 (asymmetric and symmetric C-H stretching vibration in -CH₂-), 2088 (azide), 1447, 1420 (C=C stretching vibration from ferrocene), 1380 (asymmetric deformation vibration of -CH₃ group on Si), 1360 and 1247 (symmetric -CH₃ deformation vibration), 1160 (asymmetric ring in-plane vibration for ferrocene), 1034 (asymmetric ring out-of-plane vibration for ferrocene), 892 (-CH₃ rocking vibration in S-CH₃), 865 (Si-C stretching vibration), 826 (in-plane C-H stretching in ferrocene), 771 and 742 (out-of-plane C-H deformation in ferrocene)

Synthesis of Si-PCP

3-(Trimethylsilyl)prop-2-yn-1-yl 2-chloropropionate was synthesized according to the literature.³⁹ In short: a solution of 2-chloropropionyl chloride (1.13 g, 9 mmol, 870 μl) in 20 ml THF was added dropwise to a solution of 3-(trimethylsilyl)propargyl alcohol (767 mg, 6 mmol, 897 μl) and Et₃N (907 mg, 9 mmol, 1.25 ml) in 40 ml THF under N₂ atmosphere at -20 °C. The reaction was stirred for 2 hrs at low temperature and left to warm up to room temperature. Afterwards 5 ml MeOH was added, stirring was continued for half an hour to consume the excess of 2-chloropropionyl chloride. The solvent was removed under reduced pressure, the crude product redissolved in CH₂Cl₂ and washed 2 times with saturated NH₄Cl solution and 2 times with MilliQ water. The organic layer was dried with magnesium sulfate. The solvent was removed under reduced pressure, yielding a yellowish oil which was purified using flash chromatography (heptane/EtOAc) 19:1.

¹H NMR (300 MHz, CDCl₃) of Si-PCP 0.2 ppm (s, 9H, (CH₃)₃-Si), 1.7 ppm (d, 3H, CH(Cl)-CH₃), 4.4 ppm (q, 1H, H(Cl)-CH₃), 4.7 ppm (s, 2H, -CH-CH₂-O-)

¹³C NMR (75 MHz, CDCl₃) of Si-PCP -0.3 ppm (CH₃-Si), 21.3 ppm (CH(Cl)-CH₃), 52.0 ppm (-CH-CH₂-O-), 54.1 ppm (CH(Cl)-CH₃), 93.0 and 97.9 ppm (Si-C≡C-CH₂), 169.3 ppm (-C(=O)-O-)

Synthesis of PNIPAM1 by ATRP

The initiator (**Si-PCP**) (232 mg, 1.1 mmol) was charged in an ampoule and degassed with vacuum while frozen with liquid N₂. The flask was backfilled with Ar, CuCl (106 mg, 1 mmol) was added and the system was frozen and degassed again. Me₆TREN (258 mg, 1.1 mmol, 300 μl) was dissolved in 1 ml of the solvent mixture (i-PrOH/DMF) bubbled with Ar and added to the reaction flask. After complete dissolving of the CuCl the monomer (6 g, 0.05 mol) dissolved in 8 ml (i-PrOH/DMF) was added and the reaction mixture was degassed in 3 freeze-pump-thaw cycles pump and backfilled with Ar. The reaction was terminated by exposing the mixture to air after 16 hrs. The solvent was evaporated and the polymer was redissolved in chloroform and passed through a basic alumina column in order to remove copper. The eluent was concentrated and the polymer was precipitated into diethyl ether and dried in vacuum. The polymer then was dissolved in 50 ml THF, tetrabutylammonium fluoride (156 mg) was added and stirred for overnight to remove the protecting group. THF was removed and the polymer was precipitated from chloroform to

diethylether, redissolved in water and charged in a dialysis tube (1000 Mw cut off). The polymer was dialysed till the solution become clean and transparent. The cleaned polymer was used for the click reaction after removal of water. Total yield was 2 g.

^1H NMR (300 MHz, CDCl_3) of Si-PNIPAM 0.2 ppm (s, 9H, $(\text{CH}_3)_3\text{-Si}$), 1.1 ppm (s, 6H, $-\text{CH}-(\text{CH}_3)_2$), 1.5–2.0 ppm (broad, 3H, $-\text{CH}-\text{CH}_2-$), 4.0 ppm (s, 1H, $-\text{CH}-(\text{CH}_3)_2$),

Synthesis of DDAT

S-1-dodecyl-S'-(α,α' -dimethyl- α'' -propargyl acetate) was synthesized according to the literature.³⁸ In short, S-1-dodecyl-"-(α,α' -dimethyl- α'' -acetic acid) trithiocarbonate (0.90 g, 2.5 mmol), EDC (0.96 g, 5 mmol) and DMAP (30.5 mg, 0.25 mmol) were mixed in CH_2Cl_2 (15 ml). Propargyl alcohol (0.28 g, 5 mmol) was added to the solution at 0 °C. The solution was stirred at room temperature for 48 h. The solution washed three times with MilliQ water and the solvent was removed under reduced pressure. The product was further purified with silica column chromatography (*n*-hexane and EtOAc, 1:1).

Synthesis of PNIPAM2/3 by RAFT

To obtain **PNIPAM2**, NIPAM (6.3 g, 56 mmol), **DDAT** (113 mg, 0.28 mmol), AIBN (9 mg, 0.055 mmol) and 1,4-dioxane (40 ml) were charged into a flask and purged with N_2 for one hour. The polymerization was started by heating the reaction mixture to 70 °C. The solution was stirred strongly under a small nitrogen flow for 60 minutes, to obtain low molecular weight PNIPAM. The polymerization was quenched by immersing in an ethanol/ N_2 bath (-50 °C), and warmed up to room temperature. The solution was concentrated under vacuum, and precipitated twice, into cold diethyl ether. To obtain **PNIPAM3**, the polymerization was conducted in the above detailed way, but the amount of **DDAT** (340 mg, 0.84 mmol) and AIBN (18 mg, 0.11 mmol) was increased.

^1H NMR (300 MHz, CDCl_3) of Si-PNIPAM 0.9 ppm (s, $-\text{CH}_2)_{10}-\text{CH}_3$), 1.1 ppm (s, $-\text{CH}-(\text{CH}_3)_2$), 1.25 ppm (s, $-(\text{CH}_2)_{10}-\text{CH}_3$), 1.5–2.0 ppm (broad, $-\text{CH}-\text{CH}_2-$), 4.0 ppm (s, $-\text{CH}-(\text{CH}_3)_2$), 4.7 ppm (s, $-\text{CH}_2-\text{O}-$)

ATR-FTIR of PNIPAM2/3, wavenumbers (cm^{-1}) 3289 (N-H symmetric and asymmetric stretching vibration), 3078, 2971, 2933, 2874 (asymmetric and symmetric C-H stretching vibration in $-\text{CH}_2-$), 1635 (C=O stretching vibration), 1535 (amide II), 1458, 1386, 1366 1170,

Synthesis of Bb1

PFS- N_3 (50 mg, 0.16 mmol), **PNIPAM1** (1 g), CuBr (32 mg, 0.24 mmol), PMDETA (25 mg, 0.14 mmol, 30 μl), vitamin-C (traces) were dissolved in 10 ml THF under N_2 atmosphere. The mixture was stirred for 6 weeks. The solvent was removed, the bottlebrush was dissolved in water and dialysed for 2 weeks in a membrane with 15 kDa cut off, to remove the unreacted side chains. The polymer was stored in water at 4 °C.

Synthesis of Bb2 and Bb3

PFS- N_3 (50 mg, 0.16 mmol), **PNIPAM2/3** (1.8/1 g), CuSO_4 (32 mg, 0.24 mmol), PMDETA (25 mg, 0.14 mmol, 30 μl) and 80 mg sodium ascorbate were dissolved in a mixture of 20 ml THF and 1–2 ml DMSO, under N_2 atmosphere. The mixture was stirred

for two weeks. The solvent was removed, the bottlebrush was dissolved in water and dialysed for two weeks in a membrane with 50 kDa cut off, to remove the un reacted side chains. The polymer was stored in water at 4 °C.

¹H NMR (300 MHz, CDCl₃) of Bb1,Bb2,Bb3 0.47 ppm,(s, CH₃-Si), 0.9 ppm (s, Si-CH₂-CH₂-CH₂), 1.15 ppm (br, -N-CH(CH₃)₂), 1.6 ppm (s, Si-CH₂-CH₂-CH₂), 1.9-2.2 ppm (b, -CH-CH₂-), 3.2 ppm (s, Si-CH₂-CH₂-CH₂-), 4.0 (br, -N-CH(CH₃)₂ and Cp), 4.2 ppm (s, Cp)

ATR-FTIR of Bb1,Bb2,Bb3, wavenumbers (cm⁻¹) 3289 (N-H symmetric and asymmetric stretching vibration), 3078, 2971, 2933, 2874 (asymmetric and symmetric C-H stretching vibration in -CH₂-), 1635 (C=O stretching vibration), 1535 (amide II), 1458, 1386, 1366 1170, 1037 (asymmetric ring-out-of-plane vibration for ferrocene),

Synthesis of EBDC

4-Ethynylbenzyl diethylcarbamodithioate was prepared in two steps. A solution of 3,5-dinitrobenzoyl-chloride (1.85 g, 10 mmol) in dry CH₂Cl₂ was added dropwise to a solution of 4-ethynylbenzyl alcohol (1.0 g, 7.56 mmol) and Et₃N (1.2 g, 12 mmol, ca. 1.65 ml) in dry CH₂Cl₂ (10 ml), under a slow N₂ flow at 0 °C. The solution was stirred at 0 °C for 2 hrs. Saturated NaHCO₃ (10 ml) was added to hydrolyse the excess of dinitrobenzoyl chloride. The reaction mixture was extracted with CH₂Cl₂/ aq. NaHCO₃. The organic phase was dried on Na₂SO₄ and the solvent was removed under reduced pressure. The obtained product (1-(3,5-dinitrobenzoyl)- 4-ethynylbenzoyl) was stored at -30 °C.

1-((3,5)-Dinitrobenzoyl)-4-ethynylbenzoyl (506 mg, 1.8 mmol) was reacted with sodium dithiocarbamate (440 mg, 2.0 mmol) in 7 ml DMF under Ar atmosphere. The reaction was stirred for 2 weeks at 30 °C. At higher temperature, the triple bond polymerizes. The solvent was removed under reduced pressure and the crude product was purified using column chromatography (9:1 heptane/EtOAc, silica), yielding the product as yellow crystals.

¹H NMR (300 MHz, CDCl₃) of dinitrobenzoyl-4-ethynylbenzoyl 3.15 ppm (s, 1H, HC≡C-), 3.7 and 4.05 ppm (q, 2H, N-CH₂-CH₃), 5.55 ppm (s, 2H Ar-CH₂-O-), 7.35 and 7.45 ppm (d, 4H, Ar), 9.15 and 9.25 ppm (d, 3H, Ar-N)

¹H NMR (300 MHz, CDCl₃) of EBDC 1.3 ppm (m, 6H, N-CH₂-CH₃), 3.1 ppm (s, 1H, HC≡C-) 3.7 and 4.05 ppm (q, 2H, , N-CH₂-CH₃), 4.55 ppm (s, 2H, Ar-CH₂-S-), 7.35 and 7.45 ppm (d, 4H, Ar)

¹³C NMR (75 MHz, CDCl₃) of EBDC 11.4 and 12.4 ppm (-CH₂CH₃), 41.47 ppm (Ar-CH₂-S-), 46.6 and 49.5 ppm (-CH₂CH₃), 77.3 ppm (HCCC-Ar), 83.2 ppm (HC≡C-Ar), 120.9, 129.2, 132.1 and 137.2 ppm (Ar), 194.9 ppm (-C(=S)-S-)

ATR-FTIR of EBDC, wavenumbers (cm⁻¹) 3231 (H-C≡C stretching vibration), 3040 and 3023 (Ar-H stretching vibration), 2978 and 2931 and 2910 and 2867 (symmetrical/asymmetrical stretching vibration in CH₃, -CH₂-), 1486 (C=S stretching vibration), 1452 (-CH₂- scissor vibration), 1415, 1375 (asymmetric deformation vibration of -CH₃ group), 1345, (1299), 1267 (amide II), 1208, 1141, 1092 and 1071 thiocarbamide), 1011 and 983, 918, 849 and 833 (bi-substituted aromatic C-C stretching in the benzene ring), 756, 642

Synthesis of PFS-EBDC

PFS-N₃ (56 mg, 0.18 mmol) and **EBDC** (72 mg, 0.22 mmol) were dissolved in 2 ml THF, CuBr (23 mg, 0.15 mmol) and PMDETA (0.03 ml, 0.15 mmol) were added in 1 ml THF

under N₂ atmosphere. The reaction was stirred for two weeks. The polymer was precipitated two times in MeOH.¹²

¹H NMR (300 MHz, CDCl₃) of PFS-EBDC 0.45 ppm (s, 3H, CH₃-Si), 0.85 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 1.9 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 4.2 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 3.9 and 4.15 ppm (m, 4-4 H, Cp), 1.3 ppm (m, 6H, N-CH₂-CH₃), 3.7 and 4.0 ppm (q, 4H, N-(CH₂-(CH₃)₂), 4.5 ppm (s, 2H, (Ar-CH₂-S-), 7.35 and 7.7 ppm (d, 4H, Ar), 7.6 ppm (s, 1H, N-HC=C-)

¹³C NMR (75 MHz, CDCl₃) of PFS-EBDC -3.6 ppm (CH₃-Si), 11.9 and 12.7 ppm (-CH₂CH₃), 13.3 ppm (Si-CH₂-CH₂-), 25.3 ppm (Si-CH₂-CH₂-), 53.3 ppm (-CH₂-CH₂-N), 42.1 ppm (Ar-CH₂-S-), 46.9 and 49.8 ppm (-CH₂CH₃), 69.9 ppm (Si-Cp), 71.7 and 73.6 ppm (Cp), 120.1, 126.1, 129.9, 136.2 ppm (Ar), 130.2 and 147.4 ppm (N-C(H)=C and -HC=C-Ar), 195.2 ppm (-C(=S)-S-)

ATR-FTIR of PFS-EBDC, wavenumbers (cm⁻¹) 3100, 3078 (C-H stretching vibration for ferrocene), 2972 and 2930, 2870 (asymmetric and symmetric C-H stretching vibration in CH₃, -CH₂-), 1485 (C=S stretching vibration), 1419 (C=C stretching vibration for ferrocene), 1415, 1378 (asymmetric deformation vibration of -CH₃ group), 1267, 1247 (symmetric -CH₃ deformation vibration), 1207, 1160 (asymmetric ring in-plane vibration for ferrocene), 1092 and 1072 (thiocarbamide), 1035 (asymmetric ring- out-of-plane vibration for ferrocene), 915, 892 (-CH₃ rocking vibration in Si-CH₃), 867 (Si-C stretching vibration), 828 (in-plane C-H stretching in ferrocene), 773 (out-of-plane C-H deformation in ferrocene)

Synthesis of PCP

A solution of 2-chloropropionyl chloride (1.34 g, 11 mmol, 1.07 ml) 20 ml THF was added dropwise to a solution of propargyl alcohol (0.56 g, 10 mmol, 0.58 ml) and Et₃N (1.12 g, 11 mmol, 1.53 ml) in 40 ml THF under N₂ atmosphere at -20 °C. The reaction was stirred for 2 hrs at low temperature and left to warm up to room temperature. Afterwards 5 ml MeOH was added and the solution was stirred for half an hour to consume the excess of 2-chloropropionyl chloride. The solvent was removed under reduced pressure, the crude product redissolved in CH₂Cl₂ and washed 2 times with saturated NH₄Cl solution. The organic layer was dried with magnesium sulfate and solvent was removed in vacuum, yielding a yellowish oil which was purified using flash chromatography 19:1 (heptane/EtOAc).

Synthesis of PFS-PCP

PFS-N₃ (80 mg, 0.26 mmol) and **PCP** (84 mg, 0.57 mmol) was dissolved in 10 ml THF, CuSO₄ (65 mg, 0.26 mmol) and PMDETA (52 μl, 0.26 mmol) were introduced under N₂ atmosphere, then sodium ascorbate (80 mg, 0.4 mmol) was added. The reaction was stirred for 2 weeks. Cu⁰ was removed by filtration and **PFS-PCP** was precipitated in MeOH.

¹H NMR (300 MHz, CDCl₃) of PFS-PCP 0.5 ppm (s, 3H, CH₃-Si), 0.9 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 1.9 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 4.2 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 3.9 and 4.15 ppm (m, 4-4 H, Cp), 1.7 ppm (m, 3H, CH(Cl)-CH₃), 4.4 ppm (m, 1H, CH(Cl)-CH₃), 5.3 ppm (s, 2H C-CH₂-O-), 7.6 ppm (s, 1H, N-HC=C-)

¹³C NMR (75 MHz, CDCl₃) of PFS-PCP -3.4 ppm (CH₃-Si), 13.0 ppm (Si-CH₂-CH₂-), 21.3 ppm (-CH-CH₃), 25.3 ppm (Si-CH₂-CH₂-), 52.3 ppm (-CH₂-CH₂-N), 53.0 ppm (C-CH₂-Cl), 59.0 ppm (=C=CH₂-O) 69.4 ppm (-Si-Cp), 71.5 and 73.2 ppm (Cp) 124 ppm (HC=C) 141.8 ppm (HC=C) 169.8 ppm (-C(=O)-O-)

ATR-FTIR of PFS-PCP, wavenumbers (cm^{-1}) 3140, 3083 (C-H stretching vibration for ferrocene), 2949 and 2870 (asymmetric and symmetric C-H stretching vibration in $-\text{CH}_2-$), 1741 ($\text{C}=\text{O}$ stretching vibration), 1444, 1421 ($\text{C}=\text{C}$ stretching vibration for ferrocene), 1380 (asymmetric deformation vibration of $-\text{CH}_3$ group on Si), 1360 and 1247 (symmetric $-\text{CH}_3$ deformation vibration), 1161 (asymmetric ring in-plane vibration for ferrocene), 1035 (asymmetric ring- out-of-plane vibration for ferrocene), 912, 892 ($-\text{CH}_3$ rocking vibration in $\text{S}-\text{CH}_3$), 866 (Si-C stretching vibration), 829 (in-plane C-H stretching in ferrocene), 774 (out-of-plane C-H deformation in ferrocene)

Synthesis of PFS-DDAT

PFS- N_3 (232 mg, 0.75 mmol) and **PFS-DDAT** (400 mg, 0.97 mmol) were dissolved in 30 ml THF, CuSO_4 (120 mg, 0.78 mmol) and PMDETA (157 μl , 0.78 mmol) were introduced under N_2 atmosphere, then sodium ascorbate (160 mg, 0.8 mmol) was added. The reaction was stirred for 2 weeks, then centrifuged to remove the Cu and **PFS-DDAT** was precipitated in MeOH.

^1H NMR (400 MHz, CDCl_3) of PFS-DDAT 0.45 ppm (s, 3H, $\text{CH}_3\text{-Si}$), 0.95 ppm (s, 2H, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 1.6 ppm (s, 2H, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 4.3 ppm (s, 2H, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 3.9 and 4.2 ppm (m, 4-4 H, Cp), 0.8 ppm (s, 3H, $(\text{CH}_2)_{10}\text{-CH}_3$), 1.25 ppm (s, 20H, $-(\text{CH}_2)_{10}\text{-CH}_3$), 1.7 ppm (m, 6H, $-\text{C}(\text{O})\text{-C}(\text{CH}_3)_2\text{-S-}$), 3.25 ppm (m, 2H, $\text{S-CH}_2\text{-(CH}_2)_{10}\text{-}$), 5.2 ppm (s, 2H $\text{C-CH}_2\text{-O-}$), 7.5 ppm (s, 1H, N-HC=C-)

^{13}C NMR (100 MHz, CDCl_3) of PFS-DDAT -3.52 ppm ($\text{CH}_3\text{-Si}$), 13.61 ppm ($\text{Si-CH}_2\text{-CH}_2\text{-}$), 14.3 ppm ($-(\text{CH}_2)_{10}\text{-CH}_3$), 22.90 ppm ($-(\text{CH}_2)_9\text{-CH}_2\text{-CH}_3$), 25.51 ppm ($\text{Si-CH}_2\text{-CH}_2\text{-}$ and $-\text{C}(\text{CH}_3)_2\text{-S}$), 28.06 ppm ($-(\text{CH}_2)_8\text{-CH}_2\text{-(CH}_2)_2\text{-}$), 29-31 ppm ($-(\text{CH}_2)_2\text{-(CH}_2)_6\text{-(CH}_2)_3\text{-}$), 31.98 ppm ($-(\text{CH}_2)_2\text{-(CH}_2)_9\text{-}$), 37.21 ppm ($-\text{S-CH}_2\text{-(CH}_2)_{10}\text{-}$), 53.32 ppm ($-\text{CH}_2\text{-CH}_2\text{-N}$), 56.06 ppm ($-\text{O-C}(\text{CH}_3)_2\text{-S-}$), 59.14 ppm ($=\text{C-CH}_2\text{-O-}$), 69.98 ppm (Cp-Si-), 71.89, and 73.63 ppm (Cp), 124.13 ppm (HC=C), 142.77 ppm (HC=C), 172.93 ppm ($-\text{C(=O)-O-}$).

ATR-FTIR of PFS-DDAT, wavenumbers (cm^{-1}) 3083 (C-H stretching vibration for ferrocene), 2949 and 2870 asymmetric and symmetric C-H stretching vibration in $-\text{CH}_2-$), 1728 (C(=O)-S stretching vibration), 1419 ($\text{C}=\text{C}$ stretching vibration from ferrocene), 1381 (asymmetric deformation vibration of $-\text{CH}_3$ group on Si), 1362 and 1247 (symmetric $-\text{CH}_3$ deformation vibration), 1159 (asymmetric ring in-plane vibration for ferrocene), 1034 (asymmetric ring- out-of-plane vibration for ferrocene), 888, 863 (Si-C stretching vibration), 826 (in-plane C-H stretching in ferrocene), 771 (out-of-plane C-H deformation in ferrocene)

Synthesis of PFS-N-PCP

Poly(ferrocenyl(3-aminopropyl)methylsilane) was synthesized according to the literature.^{28,43} In short: first a solution of potassium-silazide ($\text{KN}(\text{SiMe}_2\text{H})_2$) was prepared. Approximately 1.25 ml of stirred KH dispersion (0.3 g, 7.5 mmol) was washed with toluene to remove the mineral oil, 10 ml THF was added to the flask and the mixture was cooled to 0 °C. Dropwise 1,1,3,3-tetramethyldisilazane (1.0 g, 7.5 mmol, 1.33 ml) was added, and the reaction stirred for 1 hr under N_2 atmosphere. **PFS-Cl** (150 mg, 0.5 mmol) was dissolved in 3 ml THF, 18-crown-6 (0.19 g, 0.5 mmol) and 4 ml of the previously prepared solution of potassium silazide were added under N_2 atmosphere, and stirred for overnight. To remove silane protecting groups, 1 ml 0.1 M HCl solution was added and the pH was maintained around 1-2 till the PFS dissolved in the aqueous phase. The polycation was deprotonated by slowly raising the pH to 11 by adding NaOH solution. The mixture was stirred for 15

min while the polymer precipitated and 5 ml dichloromethane was added to dissolve the polymer. The two phases were separated, the organic phase was washed with NaOH solution (pH=9) and dried on Na_2SO_4 . The PFS- NH_2 solution was cooled to -70°C and Et_3N (0.15 g, 1.4 mmol, 0.2 ml) was added. Subsequently a solution of 2-chloropropionyl chloride (0.126 mg, 1 mmol, 95 μl) in 10 ml dichloromethane was added dropwise and stirred for 2 days, while allowing the solution to warm up to RT. **PFS-N-PCP** was precipitated two times into MeOH.

^1H NMR (300 MHz, CDCl_3) of PFS-N-PCP 0.45 ppm (s, 3H, $\text{CH}_3\text{-Si}$), 0.9 ppm (s, 2H, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 1.6 ppm (s, 2H, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 1.7 ppm (m, 3H, CH(Cl)-CH_3), 3.2 ppm (s, 2H, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 4.0 and 4.2 ppm (m, 4-4 H, *Cp*), 4.4 ppm (q, 1H, CH(Cl)-CH_3),

^{13}C NMR (75 MHz, CDCl_3) of PFS-N-PCP -3.4 ppm ($\text{CH}_3\text{-Si}$), 13.3 ppm ($\text{Si-CH}_2\text{-CH}_2$), 22.8 ppm ($-\text{CH-CH}_3$), 24.1 ppm ($\text{Si-CH}_2\text{-CH}_2$), 42.7 ppm ($-\text{CH}_2\text{-CH}_2\text{-N}$), 56.0 ppm ($\text{C-CH}_2\text{-Cl}$), 65.5 ppm ($-\text{Si-Cp}$), 71.3 and 73.2 ppm (*Cp*) 169.4 ppm ($-\text{C(=O)-N-}$)

ATR-FTIR of PFS-N-PCP, wavenumbers (cm^{-1}) 3290 (N-H symmetric and asymmetric stretching vibration), 3083 (C-H stretching vibration for ferrocene), 2949, 2870 (asymmetric and symmetric C-H stretching vibration in $-\text{CH}_2-$), 1655 (C=O stretching vibration), 1528 (amide II), 1441 (from PFS), 1420 (C=C stretching vibration from ferrocene), 1379 (asymmetric deformation vibration of $-\text{CH}_3$ group on Si), 1362, 1247 (symmetric $-\text{CH}_3$ deformation vibration), 1161 (asymmetric ring in-plane vibration for ferrocene), 1035 (asymmetric ring- out-of-plane vibration for ferrocene), 909, 892, 865 (Si-C stretching vibration), 828 (in-plane C-H stretching in ferrocene), 772 (out-of-plane C-H deformation in ferrocene)

Synthesis of Bb4

PFS-N-PCP (80 mg, 0.37 mmol) was dissolved in 5 ml THF and bubbled with Ar to remove oxygen from the solution. CuBr_2 (30 mg, 0.2 mmol) and a solution of NIPAM (1 g, 8.8 mmol) and Me_6TREN (80 μl , 0.4 mmol) in 5 ml THF and 1 ml DMF were added, and the mixture was further bubbled. The polymerization was started by adding vitamin-C (100 mg). The reaction was conducted for 48 hrs. The polymerization was terminated by opening the reaction vial to air. The polymer was precipitated into diethyl ether, redissolved in CHCl_3 and pressed through a basic alumina column to remove the copper. The bottlebrush was then dissolved in water and dialyzed to remove all traces of the monomer and copper. The polymer was kept in water solution.

^1H NMR (400 MHz, CDCl_3) of Bb4 0.47 ppm (s, $\text{CH}_3\text{-Si}$), 0.9 ppm (s, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 1.15 ppm (br, $-\text{N-CH(CH}_3)_2$), 1.6 ppm (s, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 1.9-2.2 ppm (b, $-\text{CH-CH}_2-$), 3.2 ppm (s, $\text{Si-CH}_2\text{-CH}_2\text{-CH}_2$), 4.0 (br, $-\text{N-CH(CH}_3)_2$ and *Cp*), 4.2 ppm (s, *Cp*)

ATR-FTIR of Bb4, wavenumbers (cm^{-1}) 3291 (N-H symmetrical/asymmetrical stretching vibration), 3078, 2967, 2926, 2872 (asymmetric and symmetric C-H stretching vibration in $-\text{CH}_2-$) 1638 (C=O stretching vibration), 1532 (amide II), 1456, ($-\text{CH}_2-$ scissor vibration), 1385, 1365, 1171, 1036 (asymmetric ring- out-of-plane vibration for ferrocene),

Synthesis of Bb 5

PFS-PCP (80 mg, 0.37 mmol) was dissolved in 5 ml THF and bubbled with Ar to remove oxygen from the solution. CuBr_2 (30 mg, 0.2 mmol) and a solution of NIPAM (1 g,

8.8 mmol) and Me₆TREN (60 μ l, 0.3 mmol) in 5 ml THF and 1 ml DMF/H₂O were added, and the mixture was further bubbled. The polymerization was started by adding vitamin-C (50 mg). The reactions was conducted for 12 hrs. The polymerization was terminated by opening the reaction vial to air. The solution was centrifuged to remove the copper, and the polymer was precipitated into diethyl ether.

Synthesis of Bb 6

NIPAM (400 mg, 2.7 mmol), **PFS-EBDC** (30 mg, 0.03 mmol), THF (3 ml) and DMF (2 ml) were charged into a quartz cuvette and purged with nitrogen for one hour. The polymerization was started by irradiation of the solution with a led photodiode ($\lambda=365$ nm) from 0.5 cm distance. The solution was shaken after every 25 minutes, and the polymerization was conducted for 4 hrs. The solution was precipitated twice, into cold diethyl ether.

Synthesis of Bb 7

NIPAM (310 mg, 3.5 mmol), **PFS-DDAT** (30 mg, 0.03 mmol), AIBN (30 μ l, 0.2 M in toluene) and 1,4-dioxane (5 ml) were charged into a flask and purged with nitrogen for one hour. The polymerization was started by heating the reaction mixture to 56 °C. The solution was stirred strongly under a small nitrogen flow for 45 minutes. The polymerization was quenched by cooling down to room temperature. The solution was precipitated twice, into cold diethyl ether.

Synthesis of Bb 8

PFS-N-PCP (80 mg, 0.37 mmol) was dissolved in 5 ml THF and bubbled with Ar to remove oxygen from the solution. CuBr₂ (30 mg, 0.2 mmol) and a solution of NIPAM (1 g, 8.8 mmol) and Me₆TREN (80 μ l, 0.4 mmol) in 5 ml THF and 1 ml DMF/H₂O were added, and the mixture was further bubbled. The polymerization was started by adding vitamin-C (100 mg). The reaction was conducted for 24 hrs. The polymerization was terminated by opening the reaction vial to air. The polymer was precipitated into diethyl ether, after drying dissolved in water and dialyzed to remove the copper salts.

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Chapter 6

Stimuli responsive properties of dual stimuli responsive molecular bottlebrushes with a redox responsive poly(ferrocenylsilane) backbone and temperature responsive poly(N-isopropylacrylamide) side chains

The redox and temperature responsive behavior of water-soluble side chain grafted poly(ferrocenylsilane)-g-poly(N-isopropylacrylamide) (PFS-g-PNIPAM) bottlebrushes were investigated. Bottlebrushes with side chains of various molar masses, 10 000 (Bb1), 19 000 (Bb2) and 2 400 (Bb4) g/mol, were synthesised as described in Chapter 5. AFM height images of Bb2 confirmed the cylindrical shape of the bottlebrush molecules. The recorded cyclic voltammograms of physisorbed bottlebrushes on a HOPG electrode in aqueous electrolyte showed two types of behavior upon oxidation-reduction. In case of Bb1 and Bb4 typical reversible, double wave oxidation curves were obtained associated with the stepwise, one-electron oxidation of the Fe atoms in the PFS backbone. Oxidation peaks were observed at $E_{ox1} = 0.39$ and $E_{ox2} = 0.56$ V and reduction peaks at $E_{red2} = 0.52$ and $E_{red1} = 0.32$ V, measured to an Ag/AgCl reference electrode. According to differential pulse voltammetry measurements, the ratio between the number of sites oxidized in the steps changed, for Bb1 from 53 to 47% and for Bb4 from 57 to 43%, as the temperature was increased from 22 to 35 °C. This indicates that the redox response is influenced by the temperature response of PNIPAM. In contrast to these measurements, Bb2 showed 'break in' behavior upon oxidation, and only one oxidation peak was observed at 0.5 V. Temperature responsive behavior of the bottlebrushes in aqueous solution was monitored by dynamic light scattering measurements. For all bottlebrushes, a lower critical solution temperature (LCST) behavior was observed. T_{LCST} was determined to be 32 °C which is identical to that of PNIPAM. The hydrodynamic diameter of the bottlebrushes decreased linearly with increasing temperature and increased linearly with decreasing temperature. Moreover, the initial size of the macromolecules was reversibly regained upon cooling. Between 20 and 32 °C the macromolecules collapsed to around 60% of their initial size. In case of Bb1 and Bb4, intramolecular collapse was observed upon crossing T_{LCST} , while Bb2 showed intermolecular aggregation, due to its longer PNIPAM grafts as compared to the other bottlebrushes.

6.1 Introduction

In recent years, macromolecules responsive to multiple types of stimuli are attracting increasing interest. Such molecules are often constructed from several building blocks where each constituent responds to a different environmental change. From a structural point of view, macromolecular bottlebrushes are polymer architectures, constructed from a backbone and polymer side chains, connected to the repeat units of the backbone. Compositional diversity of bottlebrush polymers has increased in recent years, leading to random, block or gradient copolymer main chains and side chains. Responsiveness to multiple stimuli can in general be achieved in molecular brushes by attaching different types of polymer side chains.^{1,2} with different responses or by connecting such macromolecules as copolymers.² Furthermore, in principle, both the backbone and the side chains can consist of a stimulus responsive polymer, although only few examples exist in the literature.^{3,4}

Responsiveness to variations in pH, temperature, light, magnetic field etc. are well studied in the literature for bottle brush polymers while redox responsiveness is virtually unexplored. In several examples of dual responsive systems, responses to one environmental change enhanced changes induced by other stimuli,^{1–5} usually causing emphasized collapse of the molecules as a result of increased intra- or intermolecular interactions.

We developed a macromolecular architecture based on a poly(ferrocenylsilane) (PFS) main chain and poly(*N*-isopropylacrylamide) (PNIPAM) side chains that can expand or contract in response to external stimuli. The polymeric system can be triggered through oxidation-reduction cycles involving the PFS or by temperature changes to which PNIPAM responds in aqueous environments.

Attachment of ionic side groups renders the hydrophobic PFS backbone water-soluble.⁶ Block copolymers of PFSs with water soluble polymers^{7–9} afford core-shell micelles in water, e.g. in case of PFS-co-PEG with a PFS minority block, the inner core consists of PFS chains, and the hydrophylic PEG chains are surrounding it as a corona.

PNIPAM is one of the most commonly used polymers for the preparation of temperature responsive bottlebrushes.¹⁰ In aqueous solution, PNIPAM exhibits a lower critical solution temperature (LCST) around 32 °C,¹¹ resulting in a phase transition. Upon heating, the homogeneous polymer solution becomes turbid and the polymer precipitates. The LCST of PNIPAM can be influenced/shifted by the solution composition,^{12,13} by copolymerization with other monomers^{14–17} and by the end group.¹⁸

Here we describe the responsive properties of water soluble PFS-*g*-PNIPAM molecular brushes as a function of temperature and electrochemical potential.

6.1.1 Stimuli responsive behavior of PFSs

Due to the presence of iron in the monomeric units of the polymer chain, PFS shows electrochemical properties. In addition, intermetallic coupling between the neighboring ferrocene units results in a double wave cyclic voltammogram (CV). A corresponding two-step oxidation model of PFS was proposed by Nguyen and coworkers¹⁹ and confirmed by Manners and coworkers²⁰ (Figure 6.1). In the first oxidation wave at low potential, ferrocene units at alternating positions are oxidized. The second wave is associated with oxidation of the remaining ferrocene units at higher potential.

Figure 6.1: Proposed scheme of the two step electrochemical oxidation of PFS.

Cyclic voltammetry is the most widely used electrochemical technique for acquiring quantitative and qualitative information about electrochemical reactions.²¹ In a typical CV experiment, the excitation signal is a linear potential scan with a triangular waveform, which sweeps the potential of the electrode between two switching potentials. In this process, the current is recorded as a function of the potential and

Figure 6.2: Typical double wave pattern of the PFS cyclic voltammogram [Adapted from reference²²].

shown in a cyclic voltammogram. A typical cyclic voltammogram of PFS is sketched

in Figure 6.2. The main parameters, i.e. the anodic and cathodic peak currents (i_a , i_c) and the anodic and cathodic peak potential (E_a , E_c), are labelled.

The peak current i_p (i_a or i_c in the figure) for surface-confined electroactive layers is proportional to the scan rate ν

$$i_p = \frac{nFAQ\nu}{4RT} \quad (6.1)$$

where n is the number of moles of electrons transferred, F is the Faraday constant, A is the surface area of the electrode and Q is the charge involved in the electrochemical process. If the charge transfer is slow through the surface confined layer, the system becomes diffusion controlled and the voltammetric peak current is proportional to $\nu^{1/2}$, as described by the Randles-Sevcik equation.²¹

6.1.2 PFS-g-PNIPAM molecular bottlebrushes

In Chapter 5 the synthesis of dual stimuli responsive molecular bottlebrushes consisting of PFS and PNIPAM with varying molar masses and grafting density was described (Table 6.1). The chemical composition of the bottlebrushes that will be investigated in this chapter are shown in Figure 6.3.

Figure 6.3: Chemical composition of the studied PFS-*g*-PNIPAM bottlebrushes (Bb).

Table 6.1 shows the molar mass characteristics of the bottlebrushes, based on gel

permeation chromatography (GPC) and ^1H NMR analysis. **Bb1**, **Bb2** were prepared by a 'grafting to' method, using click chemistry. **Bb4** was prepared by a 'grafting from' process where PNIPAM chains were grown from pendant ATRP initiators attached to the PFS main chain.

Table 6.1: Bottlebrushes obtained via the 'grafting to' method.

Name	PFS $M_{n,GPC}$ $\times 10^3$ g/mol	alkyne-PNIPAM $M_{n,GPC}^b$ $\times 10^3$ g/mol	alkyne-PNIPAM $M_{n,NMR}$ $\times 10^3$ g/mol	PNIPAM in Bb $M_{n,NMR}$ $\times 10^3$ g/mol	Bb $M_{n,calc}^a$ $\times 10^3$ g/mol
Bb1	169.2	11.7 ^c	10.5	4.3	4 184
Bb2	94.2	26.3	19.0	15.0	1 986
Bb4	94.2	n.a	n.a	2.4	890

^aObtained from ^1H NMR, based on PFS (Si-CH_3) and PNIPAM repeat unit integral values. ^bObtained from ^1H NMR based on alkyne- CH_2 and PNIPAM repeat unit integral values.

6.2 Results

6.2.1 AFM investigation

Following the molecular characterization of bottlebrush molecules by methods such as NMR, GPC and FTIR, atomic force microscopy (AFM) measurements were carried out which will provide information on the topology of the synthesized architectures. In case of successful 'grafting to', but especially 'grafting from' reactions, high grafting densities can be expected which directly influence the stiffness of the bottlebrushes and thereby their molecular shape.

As GPC measurements on very high molar mass, densely branched architectures may meet with interpretation difficulties as typically flexible linear polymer chains are used as standards, AFM provides important additional evidence for structural integrity of the bottlebrushes.

AFM measurements were carried out in the dry state and in solution. [Figure 6.4](#) shows (AFM) topographic images of **Bb2** deposited on a highly ordered pyrolytic graphite (HOPG) surface. The images show that molecules with a well-defined cylindrical shape were obtained in dry state and in aqueous solution (0.1 M NaClO_4). In the dry state, the average length of the molecules was 105 nm, the width was 15 nm and the height 5 nm in air. The PFS backbone has a molecular weight of around 94 200 g/mol corresponding of 303 monomer units, which should lead to a length for fully stretched molecules of 185 nm (the length of a unit is 0.6 nm²⁰). The clicked side chains were about 19 000 g/mol, thus consisting of 170 repeat units which corresponds to about 51 nm chain length (taking a unit length of 0.3 nm). In liquid environment the bottlebrush molecules retain their shape, but they swell about

3–5 times their original size in width and height. The length of the molecules doesn't change significantly. Upon heating the environment above the LCST of the PNIPAM side chains, the Bb molecules collapse, resulting in a more compact structure. The studied molecules reversibly regained about 80% of their original size upon cooling the system back to room temperature.

The response of a drop casted layer of **Bb4** molecules on HOPG to oxidation and reduction cycles was studied in situ by AFM. The freshly cleaved, atomically smooth HOPG surface is a suitable electrode in our case, since due to its hydrophobic surface, a good adhesion of the hydrophobic PFS backbone is expected.^{23,24} The obtained cyclic voltammogram, measured in situ in the AFM setup, is shown in Figure 6.5d. The HOPG substrate acted as a working-, Pt wire as a counter- and Ag wire as a reference electrode. The cyclic voltammogram shows the double oxidation and reduction waves typical of PFS.

The microscope was operated in peak force tapping mode, due to its feedback mechanism allowing one to apply low forces of around 300 pN. The resulting images, captured while a constant 0.5 V potential was applied, are presented in Figure 6.5. Image **a** was scanned from top to bottom and the marked line indicates the start of the oxidation. Image **b** was captured by scanning bottom to top and image **c** from top to bottom again. The potential was switched off at the marked line in image **c**. The marked area highlights some changes that occurred during the oxidation process. The surface morphology of the layer changed significantly, indicating swelling. As the

Figure 6.4: AFM height image of Bb2 deposited on HOPG surface, (a) in air (b) imaged in 0.1 M NaClO₄ solution at RT (Scan sizes: 1 μm × 1 μm. z-scales: 30 nm).

Figure 6.5: AFM height image of **Bb4** deposited on HOPG surface, (a - c), 0.5 V potential was applied in real time between the marked lines on (a) till (c). The highlighted area shows the morphology change of the layer. Prior to continuous oxidation a CV was recorded (d). Reference electrode Ag/AgCl, counter electrode Pt, 0.1 M aqueous NaClO_4 . Scan sizes: $1\ \mu\text{m} \times 1\ \mu\text{m}$. z-scales: 25 nm.

ferrocene units in the PFS backbone were oxidized and became positively charged, its polarity and hydrophilicity increased, which favors swelling.

6.2.2 Electrochemical response of the bottlebrushes

The electrochemical behavior of physisorbed bottlebrushes on freshly cleaved, atomically smooth HOPG surfaces was investigated with cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

Figure 6.6: Cyclic voltammograms of **Bb1** adsorbed on HOPG electrode with different scan rates at (a) 22 °C and (c) 35 °C; dependence of peak currents versus scan rates (b) 22 °C and (d) 35 °C. Reference electrode Ag/AgCl, counter electrode Pt, 0.1 M aqueous NaClO₄.

Two types of behavior were observed for the various bottlebrushes. CVs of **Bb1** and **Bb4** were recorded between 0 and 0.8 V with HOPG as a working electrode, Ag/AgCl as reference electrode and Pt as counter electrode in 0.1 M NaClO₄ electrolyte solution, at 22 and 35 °C. The process shows two reversible oxidation peaks at $E_{ox1} = 0.39$ V and $E_{ox2} = 0.56$ V and reduction peaks at $E_{red2} = 0.52$ V and $E_{red1} = 0.32$ V (Figure 6.6 and Figure 6.7), associated with the stepwise, one-electron oxidation of the Fe atoms in the PFS backbone. As the scan rate was

increased, an increase in both anodic and cathodic peak currents was measured at room temperature and at 35 °C. At the latter temperature, the bottlebrushes precipitated out of the solution. Peak currents of the two oxidation peaks plotted against the scan rate are presented in Figure 6.6b and 6.8b. The peak currents show a linear dependence against the scan rate at low temperature, indicating surface confined behavior of the bottlebrushes, in other words the polymer must be adsorbed on the electrode (HOPG) surface. At temperatures above the PNIPAM LCST there is a deviation from the linear dependency.

Figure 6.7: Cyclic voltammograms of **Bb4** adsorbed on HOPG with different scan rates at (a) 22 °C and (c) 35 °C; dependence of peak currents versus scan rates (b) 22 °C and (d) 35 °C. Reference electrode Ag/AgCl, counter electrode Pt, 0.1 M aqueous NaClO₄.

Differential pulse voltammograms recorded of the molecular brushes (Figure 6.8) provide additional insight in the stepwise oxidation of the PFS backbone. DPV curves were recorded at 5 mV/s scan rate, 50 ms pulse time, 200 ms interval time, 10 mV pulse height between 0 and 0.8 V, using HOPG as a working electrode, Ag/AgCl

reference electrode and Pt as counter electrode in 0.1 M NaClO₄ electrolyte solution. The area under the curves corresponds to the amount of ferrocene units oxidized in the steps. At room temperature in case of **Bb1**, 53% of the Fe atoms was oxidized in the first step and 57% in case of **Bb4**. Above the LCST, the ratio of the oxidation peaks changed and in the first step less than half of the ferrocene units was oxidized, namely 47% for **Bb1** and 43% for **Bb4**.

Figure 6.8: Differential pulse voltammogram recorded of **Bb1**: (a) at 22 °C (b) 35 °C and **Bb4**: (c) at 22 °C (d) 35 °, adsorbed on HOPG ($\nu = 5$ mV/s scan rate, 50 ms pulse time, 200 ms interval time, 10 mV pulse height reference electrode Ag/AgCl, counter electrode Pt, 0.1 M aqueous NaClO₄).

In contrast to **Bb1** and **Bb4**, a 'break in' behavior²⁵ was observed in case of **Bb2** upon oxidation at 22 °C (Figure 6.9). There are no double oxidation and reduction waves visible in the CV of **Bb2** and oxidation occurred at a higher potential ($E_{ox} = 0.5$ V) than found for the first wave of **Bb1** and **Bb4** ($E_{ox1} = 0.38$ V). When the PFS backbone is surrounded by a larger amount of redox inactive (isolative) polymer, the chain becomes less accessible for electron transfer. If the backbone is adsorbed via the side chains to the electrode surface and side chains prevent the

backbone and the electrode to be in close proximity, electron transfer becomes slow due to diffusion limitations caused by the PNIPAM layer. This was confirmed by the fact that upon increasing the scan rate, the PFS backbone remained unoxidized. Another difference between **Bb2** and **Bb1**, **Bb4** in their response to oxidation is that **Bb2** desorbed from the electrode surface, as was evident from the intensity of the peak current which decreased after multiple cycles.

Figure 6.9: Cyclic voltammogram of **Bb2** at 22 °C adsorbed on HOPG electrode. The cyclic voltammogram shows 'break-in' behavior and the polymer layer depletes from the surface after multiple cycles. Reference electrode Ag/AgCl, counter electrode Pt, 0.1 M aqueous NaClO_4 .

As the temperature was increased to above the PNIPAM LCST, **Bb2** precipitated. At 34 °C, the PNIPAM chains around the PFS backbone collapsed, thereby also forming a more dense layer between the electrode surface and the backbone. Electron transfer to the PFS backbone likely became more difficult and a CV could not be measured.

6.2.3 Temperature responsiveness of the bottlebrushes studied by dynamic light scattering

Light scattering measurements constitute a powerful method for acquiring the average diameter of the synthesized bottlebrush molecules in water, *i.e.* in their solvated state. By performing the measurements as a function of temperature, dimensional changes induced by temperature variation can be monitored directly. Dynamic light scattering (DLS) measurements were carried out in aqueous solution at concentrations of around 0.5 w/w%. A laser with a wavelength $\lambda = 632$ nm was used and the solution temperature was controlled within ± 0.1 °C. From the obtained correlation function, the average hydrodynamic diameter (D_h) of the molecular bottlebrush molecules was calculated.

Temperature scans were carried out by increasing/lowering the temperature stepwise with increments of 0.3 °C. After each step, the samples were equilibrated for

Figure 6.10: Dependence of the hydrodynamic diameter D_h on temperature: during multiple cycles from 28 to 34 °C for (a) **Bb1** and (c) **Bb4**. Behavior over a wider temperature range: (b) **Bb1**, (d) **Bb4** and (e) **Bb2**.

two minutes. For all bottlebrushes, D_h decreased with increasing temperature and increased with decreasing temperature. Moreover, the initial size of the macro-

molecules was reversibly regained upon cooling (Figure 6.10). The LCST temperature was determined from the graphs to be 32 °C, since this was the temperature at which the scattering intensity increased sharply (data not shown). The size of **Bb1** and **Bb4** continued to decrease above the LCST temperature, while **Bb2** showed aggregation. The largest observed size reductions were from 45 to 32 nm (**Bb1**), 110 to 75 nm (**Bb4**) and 150 to 110 nm (**Bb2**) between 25 and 32 °C. Figure 6.10a and 6.10c show that the 'original' D_h is recovered following two temperature cycles. Here the temperature was stepped with increments of 0.8 °C between 28 and 34 °C. We note that as the scattering angle was 173°, the data must be treated carefully. For quantitative interpretation, measurements at low angles have to be performed.

6.3 Discussion

The various PFS-*g*-PNIPAM bottlebrushes show both redox and temperature responsiveness. Their behavior, however, is also influenced strongly by their architecture.

Figure 6.11: Schematic drawing of the structures of the investigated PFS-*g*-PNIPAM bottlebrushes, estimated from the molar mass characteristics.

Figure 6.11 shows a schematic representation of the molecules, based on the NMR, GPC and DLS measurements. **Bb1** has a low grafting density, and a long backbone with relatively long side chains. **Bb4** has a somewhat shorter backbone, but its grafting density is unknown. Assuming a perfect initiation it bears short, densely packed side chains. **Bb2** has the same backbone length as **Bb4**, yet it possess long, densely grafted side chains. DLS measurements showed that the highest apparent $D_{h,max} = 150$ nm hydrodynamic diameter belonged to **Bb2** which is expected, as

it has the longest side chains. The most compact structure was found to be **Bb1**, which was almost half the size of **Bb4**. Since the side chains are further away from each other the PFS backbone is more flexible, and more inclined to shield its hydrophobic backbone from the aqueous environment. Likely, **Bb1** forms a collapsed globular conformation in water. The dimensions of the bottlebrushes obtained by AFM matched well with those obtained by dynamic light scattering.

Each Bb collapsed to 70% their original size upon increasing the temperature from 25 to 32 °C. The LCST temperature was found to be around 32 °C in all cases, but different types of LCST behavior were observed. Previously Matyjaszewski and coworkers²⁶ showed for temperature responsive bottlebrush molecules, that due to the compact structure of molecular brushes, intramolecular collapse can occur when the average distance between molecules is much larger than the hydrodynamic dimensions of the individual macromolecules. However, if the concentration of the solution of molecular brushes is increased to the level in which the separation distance is comparable with the brush hydrodynamic dimensions, intermolecular aggregation occurs, as typically observed for solutions of linear polymers.²⁷ In another study on poly(thiophene)-*g*-PNIPAM,³ a twofold decrease in size was found accompanied by a coil-to-collapsed globule behavior, but no intermolecular aggregation occurred. Similar to our bottlebrushes, the poly(thiophene)-*g*-PNIPAM brushes had a side chain length of 1000-5000 amu in weight. In our case **Bb1** and **Bb2** show a continuous intramolecular collapse above the LCST, while **Bb2** aggregates. This difference is therefore caused by the longer side chains and even at low concentration, intermolecular aggregation occurs.

The size of the macromolecules decreased essentially linearly until the LCST, and the same size was recovered after cooling at the applied heating/cooling rate. Collapse over a wide temperature range was found in several cases of PNIPAM bottlebrushes,^{28–30} resulting in a 10–20% decrease in size. Whittaker and coworkers³¹ observed a linear size change for PNIPAM star polymers.

Figure 6.12: Schematic drawing of the PFS-*g*-PNIPAM bottlebrush molecule deposited on a HOPG surface. Long chains of PNIPAM form an isolating layer between the electrode surface and the bottlebrush backbone and screen the electron transfer.

The redox properties of the PFS main chain were found to depend on the bottlebrush structure. Typical double wave CVs were obtained for **Bb1** and **Bb4**. For **Bb1** with its long PNIPAM side chains, the two oxidation and reduction waves could not be resolved, especially at higher scan rates. This likely is due to slow electron transfer.³² As is known for PFS, half of the ferrocene units are oxidized in the first step and the remaining half in the second, therefore the ratio of the two peaks should be 1:1.¹⁹ DPV curves obtained at 5 mV/s scan rate for **Bb1** and **Bb4** show that below the LCST more than the half of the ferrocene sites are oxidized, while above it the ratio changes. The peak current below the LCST shows a linear dependence on the scanning rate, indicating typical surface-confined electron transport. However above the LCST neither the scan rate (ν) nor its square root ($\nu^{1/2}$) scales linearly to the peak current, as would be the case for pure diffusion-controlled electron transport, showing the insulating effect of the collapsed side chains.

These result suggest that first of all, the ferrocene units are in close proximity to the HOPG electrode surface and although water is a nonsolvent for the PFS backbone, it becomes accessible to the supporting electrolyte ions because of the hydrophilic PNIPAM side chains, similarly as reported previously for water soluble PFS polyion multilayers on gold.³³ Secondly, above the LCST of PNIPAM, electron transport is hindered due to the collapse of the PNIPAM chains, now acting as an insulator (Figure 6.12).³⁴ Therefore, the bottlebrush architecture plays an important role in the oxidative response. **Bb2** possesses rather long and densely packed PNIPAM side chains compared to **Bb1** and **Bb4**, forms a loose layer on the electrode surface and, upon oxidation, departs from the surface. At low scan rates (1-10 mV/s), break in behavior is observed for **Bb2** and a single oxidation peak is found at higher potentials adsorbed from aqueous solution.

6.4 Summary and Conclusion

Water soluble PFS-*g*-PNIPAM bottlebrushes were imaged by atomic force microscopy. Height images of **Bb2** molecules deposited on a HOPG surface were obtained in air and in 0.1 M aqueous NaClO₄. These images confirmed the cylindrical shape of the bottlebrush molecules. In the dry state, the average length of the molecules was 105 nm, the width was 15 nm and the height 5 nm. In liquid environment the bottle brush molecules retained their shape, but they swelled about 3-5 times their original size in width and height. The length of the molecules did not change significantly. The PFS-*g*-PNIPAM bottlebrushes showed dual stimuli responsive behavior. Both electrochemical and temperature responses of the bottlebrushes were influenced by the length of the side chains. The recorded cyclic voltammograms of physisorbed bottlebrushes on HOPG electrodes in aqueous NaClO₄ showed two types of behavior upon oxidation-reduction. In case of **Bb1** and **Bb4** typical reversible double-wave oxidation curves were obtained associated with the stepwise, one-electron oxidation of the Fe atoms in the PFS main chain. According to the differential pulse voltammetry

measurements, the ratio between the amount of sites oxidized in the steps changed, for **Bb1** from 53 to 47% and for **Bb4** from 57 to 43%, upon increasing the temperature from 22 to 35 °C, indicating the influence of the temperature responsiveness of the PNIPAM grafts. In contrast to these measurements, **Bb2** showed a 'break in' behavior upon oxidation and only one oxidation peak was observed at 0.5 V. The thermoresponsive behavior of the bottlebrushes in aqueous solution was also monitored by dynamic light scattering measurements. For all bottlebrushes, lower critical solution temperature (LCST) behavior was observed, T_{LCST} was determined to be 32 °C, which is identical to the PNIPAM LCST. DLS measurements showed that the highest apparent $D_{h,max} = 150$ nm hydrodynamic diameter belonged to **Bb2** which is expected, as it has the longest side chains. The most compact structure was found to be **Bb1** ($D_{h,max} = 45$ nm), which has the lowest grafting density. Since the side chains are further away from each other the PFS backbone is more flexible, and more inclined to shield its hydrophobic backbone from the aqueous environment. The hydrodynamic diameter of the bottlebrushes decreased continuously with increasing temperature and continuously increased with decreasing temperature. Moreover, the initial size of the macromolecules was reversibly regained upon cooling. Likely, the bottlebrushes go from an extended to a collapsed globular conformation in water, to shield their hydrophobic backbone from the aqueous environment. The dimensions of the bottlebrushes obtained by AFM matched well with those obtained by dynamic light scattering.

6.5 Experimental section

Materials

Chemicals were purchased from Sigma-Aldrich (The Netherlands) and used as received. Organic solvents were purchased from Biosolve (Valkenswaard, The Netherlands). Deionized water from a MilliQ Advantage A 10 purification system (Millipore, Billerica, Ma, USA) was used.

Methods

Dynamic light scattering

DLS measurements were carried out with a Malvern NanoS equipped with 632 nm wavelength laser, the scattered light was detected at 173° angle. The temperature was changed from 16 to 40 °C with 0.1 °C precision. In each temperature the polymer was equilibrated for two minutes, and each measurement point was an average of 10 scans. The data was analysed with Malvern analysis software.

Electrochemical measurements

Electrochemical measurements were carried out on an AutoLab PGSTAT 10 electrochemical workstation. HOPG was used as a working-, and Pt wire as counter electrode against an

Ag/AgCl reference electrode. Voltammograms were recorded at different scan rates 10, 20, 50 and 100 mV/s and between 0 and 0.8 V. Differential pulse voltammograms were measured with $\nu = 5$ mV/s scan rate, 50 ms pulse time, 200 ms interval time, 10 mV pulse height.

Atomic Force Microscopy

A Bruker Multimode AFM with a heater-cooler setup and a NanoScope V controller was used for the variable temperature measurements equipped with a closed quartz liquid cell as a tip holder for the liquid measurements (Bruker/Veeco/Digital Instruments, Santa Barbara, CA).

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Chapter 7

Towards an adhesion driven molecular crawler; synthesis of an asymmetrical dual stimuli responsive molecular bottlebrush

*A dual stimuli responsive molecular gradient bottlebrush was obtained using 'grafting to' processes. As a backbone redox responsive poly(ferrocenylsilane) (PFS) with a composition gradient and as side chains temperature responsive poly(*N*-isopropylacrylamide) (PNIPAM) were used. PFS with a composition gradient was obtained via platinum catalyzed ring-opening polymerization of [1]silaferrrocenophane monomers featuring ethyl-methyl or methyl-chloropropyl groups at the silicon atom by controlling the feed ratio. After polymerization the chloropropyl moieties were converted into azidopropyl groups. According to ^1H NMR measurements the final product contained 35% azide groups and 65% of inert ethyl-methyl substituted units. The obtained monomodal peak in gel permeation chromatography traces and the steadily increasing molar mass with time confirmed that copolymerization of the monomers occurred. The 'grafting to' method involved a Huisgen cycloaddition click reaction between an azide functionalized PFS backbone and alkyne end-functionalized PNIPAM chains. Based on ^1H NMR- and ATR-FTIR spectroscopy we estimate that the click reaction between the backbone and the side chains afforded a gradient bottlebrush with a molar mass of $M_n = 2.7$ million g/mol. In the click reaction, approximately 75% of the side chains were functionalized with PNIPAM, yielding a bottlebrush with a relatively high grafting density. Dual stimuli responsive properties of the gradient bottlebrush were assessed by cyclic voltammetry (CV) measurements. In organic electrolyte, reversible double wave oxidation curves were obtained associated with the stepwise, one-electron oxidation of the Fe atoms in the PFS backbone. In aqueous solution, CVs were recorded below and above the critical solution temperature of the PNIPAM. At 22 °C three oxidation peaks at 0.44, 0.48 and 0.53 V and two reduction peaks at 0.5 and 0.38 V were observed, while at 38 °C a double wave with small separation between the oxidation peaks (0.41 and 0.46 V) were found, influenced by the isolative nature of the PNIPAM. Due to the stepwise decay of the side chains along the backbone, the redox centers experience varying environments within a bottlebrush molecule, leading to multiple redox events.*

7.1 Introduction

Introducing directed molecular motion of macromolecules, deposited on surfaces, is an essential requirement for targeted delivery in nanoscale devices,^{1,2} however it is a challenging task to achieve directional translational motion at the molecular level. There are several examples in the literature where motion was induced thermally, and guided by the lattice of the underlying surface.^{3–5} Stimuli responsive polymers are principal candidates in studies aimed at achieving directed molecular motion. Among these, stimuli responsive macromolecular bottlebrushes are of special interest as they can be triggered externally to change their conformation, and can be tracked by atomic force microscopy.^{6–9} Dual responsive molecular bottlebrushes based on a redox responsive poly(ferrocenylsilane) (PFS) backbone and temperature responsive poly(*N*-isopropylacrylamide) (PNIPAM) side chains can be rearranged on a surface by applying an external stimulus. In principle, surfaces possessing a gradient in properties such as wettability may induce motion of triggered molecules in a directed way.

For this objective we discuss the synthesis of an unsymmetrical bottlebrush based on PFS and PNIPAM chains. To tune the grafting density of the sidechains, a composition gradient was introduced along the backbone from a further functionalizable monomer and a nonfunctionalizable, diluent monomer.

As discovered by the Manners group, [1]silaferrocenophane monomers undergo ring-opening polymerization (ROP),¹⁰ driven by the significant ring strain present in these cyclic monomers. The [1]silaferrocenophanes consist of a ferrocene where the cyclopentadienyl rings are bridged by a silicon atom that possesses two additional pendant groups R and R'. The polymerization reaction can be conducted either by using anionic initiators or by adding transition metal catalysts.^{11,12} In principle both methods are appropriate for preparing copolymers from different silaferrocenophane monomers. However, many R groups do not tolerate the high basicity and reactivity of the anionic initiators or of the living PFS chain end, thus limiting the choice of R and R'. Recently, the Manners groups described photoassisted living anionic ring opening polymerization, requiring milder initiators and thereby broadening the range of anionically polymerizable monomers.¹³ Here we chose to use platinum-catalysed ROP as it leads to high molar mass PFSs and is compatible with our functional side groups.^{12,14–18}

Copolymers featuring a gradient in composition are well known and many examples can be found in the literature.^{19–23} Gradients in composition of an AB copolymer can be achieved if the monomers A and B undergo copolymerization, the chain growth is not interrupted by termination or chain transfer reactions, and if the final copolymer composition depends on the feed ratio of its constituents A and B. One option for obtaining a gradient is the continuous introduction of monomer A into a polymerizing mixture of A and B, resulting in a gradual decrease in the concentration of B as chain growth progresses.

To obtain a dual stimuli responsive gradient molecular bottlebrush, PNIPAM side chains were attached to a gradient PFS backbone in a 'grafting to' process. The

method involved a Huisgen cycloaddition click reaction between an azide functionalized PFS backbone and alkyne end-functionalized PNIPAM chains.^{24,25} The dual stimuli responsive nature of the macromolecule was assessed by electrochemical measurements.

7.2 Results

7.2.1 Synthesis of a gradient PFS backbone

To obtain PFS chains with a gradient in composition, two different ferrocenophane monomers were copolymerized: [1](3-chloropropyl)methylsilaferrocenophane (**SF-PrCl**) and [1]ethylmethylsilaferrocenophane (**SF-Et**) (Scheme 7.1). Based on reports that [1]silaferrocenophanes can be copolymerized with other strained, cyclic alkylsilanes²⁶ in the presence of platinum catalysts, we concluded that copolymers of [1]silaferrocenophanes can be formed by ring opening polymerization. A step-wise decay in the concentration ratio $[\text{SF-PrCl}]/[\text{SF-Et}]$ was achieved by controlling monomer feed. Sequential introduction of the monomers to the polymerization solution resulted in poly(ferrocenyl(3-chloropropyl)methylsilane)-*grad*-poly(ferrocenylethylmethylsilane) (**grPFS-Cl**).

Scheme 7.1: Synthesis of the composition gradient PFS, consisting of **grPFS-Cl** and **SF-Et** monomer units.

The Pt-catalyzed ring opening polymerization was conducted as follows: the polymerization was started with **SF-PrCl**, forming a pure **SF-PrCl** block. Polymerization was allowed to proceed for 1 hr and then **SF-Et** monomer was introduced after a sample (**S1**) was taken for analysis. After another hour a new sample was taken (**S2**) and again **SF-Et** was added to the solution. After 1 hr a third sample was taken (**S3**) and **SF-PrCl** was added. The polymerization was continued for another 5 hrs (total 8 hrs) to obtain the gradient polymer (**grPFS-Cl**). Each sample and also the final product were precipitated in MeOH. The samples were characterized by means of ^1H NMR spectroscopy and GPC to gauge the progress of the polymerization. ^1H NMR spectra recorded from each sample (Figure 7.1) show how the polymer composition evolves with the proceeding reaction. The chemical shift of the signal belonging to the methyl group at the silicon atom differs for the two different repeat

Figure 7.1: ^1H NMR spectra of the samples, 1-4 from bottom to top, the magnified part shows the ratio of the two different repeat units in the PFS chain.

units. Therefore the integrals' ratio of these two singlets ((O1, O2) in Figure 7.1) gives information about the composition of the polymer. S1 contains only SF-PrCl units which feature a peak at $\delta = 0.52$ ppm (O1). The relative amount of SF-PrCl decays from sample to sample. The final product (grPFS-Cl) possesses around 35% SF-PrCl and 65% SF-Et units, which is in agreement with the feed ratio. Since there is a relatively long block in the beginning of the chain, the content of SF-PrCl units is less than 50% after introducing the other monomer.

Figure 7.2: GPC traces of the samples taken during the polymerization of **grPFS**. The peaks shift to lower elution volume as the polymerization proceeds.

To ensure that polymerization proceeded during the course of the experiment, GPC measurements were carried out for each sample (Figure 7.2). The obtained traces show a monomodal distribution for each sample and a peak maximum that shifted to lower elution volumes from **S1** to the final product. The polystyrene-effective molar masses and polydispersities obtained for the samples are summarized in Table 1. The relative increase in the molar mass decreases, showing that the polymerization rate decreases in time, likely due to a higher viscosity caused by the growing polymer. The polydispersity of the samples increased from 1.82 to 2.65, which are typical values for PFSs obtained by Pt-catalyzed ring-opening polymerization.

Table 7.1: Molar mass characteristics of the gradient PFSs.

Name	M_n^a 10 ³ g/mol	M_w 10 ³ g/mol	PDI	M_w^b 10 ³ g/mol
S1	99.2	180.3	1.82	181.3
S2	126.4	286.3	2.27	210.9
S3	130.5	301.8	2.31	220.6
grPFS-CI	142.0	376.1	2.65	247.8

^aMeasured in THF against narrow PDI polystyrene standards.

^b Peak molar mass.

7.2.2 Azide functionalized gradient backbone

To obtain a molecular bottlebrush, the PFS backbone was functionalized with pendant azide groups for further derivatization using click chemistry (Azide-alkyne Huisgen cycloaddition). To introduce azide-functional pendant groups, **grPFS-Cl** was reacted with NaN_3 to yield poly(ferrocenyl(3-azidopropyl)methylsilane)-*grad*-poly(ferrocenylethylmethylsilane) (**grPFS-N₃**).²⁷

Figure 7.3: ^1H NMR spectrum of the azide functionalized grPFS. The conversion of the side group is evident by the shift of the peaks belonging to the pendant groups attached to Si. For guidance to the symbols **J** and **K** see [Scheme 7.2](#).

^1H NMR spectroscopy shows a quantitative conversion of **grPFS-Cl** to **grPFS-N₃**. This can be judged by the shift of the 3-CH₂ signal (**J**), as it depends directly on the attached group, Cl or N₃. To ensure the absence of chain scission during the side group conversion, GPC measurements were performed (Figure 7.4). The shapes of the peaks were similar but the **grPFS-N₃** peak shifted to higher elution volume with respect to the **grPFS-Cl** signal, suggesting a lower molar mass for **PFS-N₃**. However, as the polydispersities are similar and no obvious signals belonging to low molar mass polymer chains were present, chain scission can be ruled out. The lower polystyrene-effective molar mass of **PFS-N₃**, compared to the **grPFS-Cl** most likely is caused by a smaller hydrodynamic volume of the PFS-N₃ coil due to dipolar intramolecular interactions between its azide moieties.

Figure 7.4: GPC traces of **grPFS-Cl** (black) and its derivative **grPFS-N₃** (grey).

7.2.3 Synthesis of a gradient molecular bottlebrush via a 'grafting to' method

A bottlebrush backbone, functionalized with azido moieties in a composition gradient, is now available for attaching PNIPAM chains, end-functionalized with an alkyne group. Well-defined PNIPAM chains were obtained, by the reversible addition-fragmentation chain-transfer (RAFT) process.²⁸

The alkyne group was introduced via an alkyne-bearing chain-transfer agent (S-1-dodecyl-S'-(α,α' -dimethyl- α'' -propargyl acetate) for RAFT polymerization. The RAFT process was conducted according to a literature procedure where the CTA concentration was adjusted to control the molar mass. PNIPAM chains of $M_n = 26.3 \times 10^3$ g/mol were obtained as established by GPC and end-group analysis using

Scheme 7.2: **grPFS-Cl** was derivatized to **grPFS-N₃**, to which alkyne end-functional PNIPAM was attached via click chemistry, yielding the **grBb**.

¹H NMR spectroscopy (for detailed characterization of the polymer see Chapter 5, **PNIPAM2**).

The click reaction (Scheme 7.2) which attaches the PNIPAM chains to the PFS backbone was conducted in tetrahydrofuran containing 5% dimethylsulfoxide in the presence of Cu⁰/Cu^I catalyst system, that was produced by in situ reducing Cu^{II} to Cu⁰ with sodium-ascorbate.²⁹ To remove the reduced Cu after clicking, the solution was centrifuged. Subsequently, unreacted PNIPAM chains were removed by dialysis in methanol using a large-pore dialysis tubing. The solubility of the product in methanol already gave an indication of the successful PNIPAM attachment to the hydrophobic PFS backbone.

ATR-FTIR spectroscopic characterization of the gradient bottlebrush is presented in Figure 7.5. First of all, ATR-FTIR spectra clearly show the successful derivatization of **grPFS-Cl** to **grPFS-N₃** by the appearance of the azide stretching peak at 2088 cm⁻¹.³⁰ Importantly, the absence of this peak in the spectrum of the **grBb** provides evidence for the successful click reaction of the polymers. Furthermore, the characteristic PNIPAM amide absorptions at 1635 and 1535 cm⁻¹ and the characteristic peak at 1036 cm⁻¹ belonging to the out-of-plane vibration of the ferrocene rings show that both polymers are incorporated in the bottlebrush. The gradient bottlebrush shows a higher PFS content than the previously made, homogeneous bottlebrushes.

Thermogravimetric analysis confirms the high PFS content of the gradient bottlebrush. The decomposition of **PFS-N₃** and PNIPAM follows a different pathway. PNIPAM is stable up to 380 °C and after decomposition 1.0 and 0.6 wt.% remains.

Figure 7.5: (a) ATR-FTIR spectra of the **grBb**, **grPFs-Cl**, **grPFs-N₃** and the alkyne-bearing **PNIPAM**. The completion of the click reaction is apparent from the absence of the azide stretching peak at 2088 cm⁻¹ in the spectrum of **grBb**. (b) Magnification of (a) the region between 1300 and 900 cm⁻¹, the absorbance at 1036 cm⁻¹ in the spectrum of **grBb** belonging to the out-of-plane vibration of the ferrocene rings, is absent from the spectrum of **PNIPAM**.

The gradient bottlebrush molecule followed a similar decomposition path as the **PNIPAM**, but resulting in a higher residual weight. The **grBb** decomposed in two steps,

Figure 7.6: Thermal decomposition of the **grBb** molecule, as a reference **PFS-N₃**, the **PNIPAM** side chain and **Bb2** are shown. The inset is a magnification between 400 and 600 °C.

losing 5-10 wt.% at 150 °C and most of its weight at 350 °C, resulting in 5.6% residual weight. Decomposition of **PFS-N₃** occurred at 250 °C and its 43% residual weight originated from the remaining iron and silicon, which are also responsible for the residual weight of the bottlebrush. For comparison the decomposition of bottlebrush **Bb2** is shown in Figure 7.6. This bottlebrush consists of a homogeneous backbone (**PFS-N₃**) and **PNIPAM** side chains, branching from each monomer unit. This bottlebrush decomposed in two steps as well, losing 5% weight at 150 °C and showing a strong weight loss at 345 °C, resulting in a lower, 2.8% residual weight.

7.2.4 Electrochemical response of the gradient bottlebrush

The stimuli responsive behavior of the gradient molecular brush was studied by electrochemical measurements. The bottlebrush was physisorbed on an electrode surface and investigated with cyclic voltammetry (CV) in organic as well as in aqueous solution. Due to the presence of Fe in the repeat units of the polymer chain, PFS shows a distinctive electrochemical response.^{31,32} PNIPAM exhibits a lower critical solution temperature (LCST) in aqueous solution at 32 °C, above which the polymer precipitates from the solution.

The polymer was deposited on the surface of a glassy carbon electrode by dipping it several times in a solution of the **grBb** in methylene chloride followed by drying under vacuum. CVs were recorded between -0.1 and 0.6 V in 0.1 M NBu₄PF₆ in acetonitrile solution, with the glassy carbon as a working-, Ag/Ag⁺ as a reference- and Pt as a counter electrode, using a scan rate of 50 mV/s. A double wave oxidation

Figure 7.7: Cyclic voltammogram of **grBb** recorded between -0.1 and 0.6 V in acetonitrile with 0.1 M NBu_4PF_6 as electrolyte. A supporting glassy carbon electrode was used as working electrode, Ag/Ag^+ as a reference- and Pt as counter electrode.

curve was observed, showing the typical two-step oxidation process of PFSs (Figure 7.7). The two reversible oxidation peaks were determined to be at: $E_{o1} = 0.22$ V, $E_{o2} = 0.45$ V and the reduction peaks are at $E_{r2} = 0.37$ V and $E_{r1} = 0.15$ V. The polymer layer was stable only through a few cycles on the electrode surface, after which it depleted.

In aqueous solution CVs were recorded as the potential was swept between 0 and 0.6 V at 22 and 38 °C. As a working electrode glassy carbon was used, while Ag/AgCl served as a reference- and Pt as a counter electrode. The deposited polymer layer remained attached to the surface even after several scans. The CV showed reversible oxidation and reduction peaks. At 22 °C, below the LCST temperature of PNIPAM, three oxidation peaks (E_o) at 0.44, 0.48 and 0.53 V and two main reduction peaks (E_r) at 0.5 and 0.38 V could be distinguished, while above the LCST a double wave, with a small separation between the oxidation peaks at 0.41 and 0.46 V, and reduction peaks at 0.5 and 0.4 V were found (Figure 7.8). To gain more insight in the oxidation process, differential pulse voltammograms were recorded at 22 and 38 °C, with a scan rate of 5 mV/s, 50 ms pulse time, 200 ms interval time, 10 mV pulse height between 0.2 and 0.6 V, in 0.1 M NaClO_4 electrolyte solution. At both temperatures two main oxidation peaks were observed (Figure 7.8), possibly because of the slower scan rate. At room temperature the oxidation peaks were at 0.41 and 0.53 V while above the LCST they were at 0.42 and 0.54 V. The area under the curves corresponds to the amount of ferrocene units oxidized in the steps. It was not possible to split the curves accurately into two peaks, corresponding to the number of electron transfer steps. In each case, the best fits obtained showed an equal amount

Figure 7.8: Cyclic voltammograms of **grBb** adsorbed on a GC electrode with 50 mV/s scan rate at (a) 22 °C and (c) 38 °C. Differential pulse voltammogram recorded (b) at 22 °C and (d) 38 °C (reference electrode Ag/AgCl, auxiliary electrode Pt, 0.1 M NaClO₄ electrolyte solution).

of transferred electrons.

7.3 Discussion

PFS with a composition gradient was synthesized via ring-opening copolymerization of [1]silaferrocenophane monomers featuring ethyl-methyl or methyl-chloropropyl groups at the silicon atom. After polymerization, the chloropropyl moieties were converted into azidopropyl groups, allowing further modification by click chemistry. The azide-alkyne click reaction was employed to attach alkyne-end-functional PNIPAM chains to the **grPFS** backbone, forming a gradient bottlebrush molecule. Based on ¹H NMR spectroscopic analysis the backbone contained 35 mol% of functionalizable

monomer. According to GPC measurements the molar mass of the backbone was $M_n = 142\,000$ g/mol. Based on the monomer composition, 170 repeat units of the bottlebrush bear a PNIPAM chain. Regarding the integral values of the Si-CH₃ groups from PFS and the PNIPAM repeat units in the ¹H NMR spectrum of **grBb**, its molar mass was estimated to be $M_{n,calc} = 142\,000 + 170 \times 15\,000 = 2.692 \times 10^6$ g/mol. Since the effective fractionation range of the GPC columns extends to around 2 million g/mol, the gradient bottlebrush was not analyzed by GPC.

Table 7.2: Molar mass characteristics of the gradient bottlebrush polymer.

Name	PNIPAM $DP_{n,NMR}$	PNIPAM $M_{n,NMR}$ 10^3 g/mol	$M_{n,calc}$ 10^6 g/mol
grBb	133	15.0 ^a	2.692

^aObtained from ¹H NMR, based on PFS (Si-CH₃) and PNIPAM repeat unit integral values.

The cyclic voltammograms and differential pulse voltammograms of the gradient bottlebrush showed characteristics typical of PFSs. In organic media, due to the high solubility of both constituent polymers, two well-separated oxidation and reduction peaks were observed, indicating efficient counterion transport to the backbone. For sufficient charge transport to and from the PFS backbone, it has to be accessible to the supporting electrolyte ions. As previously shown in case of homogeneous bottlebrushes (see Chapter 6), long PNIPAM grafts hinder electrolyte diffusion, resulting in a break-in behavior upon oxidation.³³

Even though a relatively large amount of PNIPAM was attached to the backbone, the bottlebrush was not soluble in water. Therefore, direct temperature responses of the PNIPAM side chains, studied for instance by monitoring the size change of the bottlebrush at different temperatures, were not investigated. Nevertheless, electrochemical measurements in 0.1 M NaClO₄ showed influence of the presence of the PNIPAM grafts on the stepwise oxidation behavior of the gradient bottlebrush.

Since there is an inhomogeneity in the backbone, the backbone is differently exposed to the solvent. The majority of the repeat units in the PFS backbone are hydrophobic, and only about 35% of them bear a PNIPAM side chain, stepwise decaying along the backbone, thus the backbone has a purely hydrophobic tail. Due to this gradient composition the accessibility of the redox centers in the chain varies, resulting in an apparent multiple step electron transfer. Therefore at 50 mv/s scan rate several oxidation peaks can be distinguished from the CV curve. Above the LCST, however, due to the non-solubility of the PNIPAM chains, the oxidation peaks are not separated from each other, showing the influence of the isolative nature of collapsed PNIPAM.³⁴ The different behavior of the bottlebrush molecules in organic and aqueous solution is well known from previous studies of surface grafted PFS

Figure 7.9: Schematic representation of the bottlebrush with a grafting density decay along the side chain.

layers.³⁵ There, unsymmetrical oxidation peaks were observed in aqueous solution, due to the low accessibility of the hydrophobic chains, although in organic electrolyte symmetrical, well-separated peaks were recorded. In our case according to the differential pulse voltammograms, due to the presence of the PNIPAM grafts, at low scan rate, the amount of transferred electrons in both redox steps is roughly equivalent.

This low solubility of the macromolecule in aqueous environment is probably due to the inhomogeneous distribution of the water soluble PNIPAM chains along the backbone. As the electrochemical response indicates a certain state of solvation, the formed polymer brush might behave similarly as PFS block copolymers possessing a water-soluble block, where due to favorable interactions between the bare PFS segments micellization occurs in aqueous solution.^{16,36,37}

7.4 Summary and Conclusion

A dual stimuli responsive gradPFS-*g*-PNIPAM molecular gradient bottlebrush was obtained using 'grafting to' processes. PFS with a composition gradient was obtained via platinum catalysed ring-opening polymerization of [1]silaferrocenophane monomers featuring ethyl-methyl or methyl-chloropropyl groups at the silicon atom by controlling the feed ratio. After polymerization the chloropropyl moieties were converted into azidopropyl groups. According to ¹H NMR measurements, the final product contained 35% of azide groups and 65% of the inert ethyl-methyl substituted units. The obtained monomodal peak in gel permeation chromatography traces and the continuously increasing molar mass during polymerization confirmed the copolymerization of the monomers. The click reaction between the azide functionalized PFS backbone and the alkyne end-functionalized PNIPAM chains afforded a gradient bottlebrush with a molar mass of $M_n = 2.7$ million g/mol. Dual stimuli responsive properties of the gradient bottlebrush were assessed by cyclic voltammetry measurements. In aqueous solution the shape of the recorded CVs was influenced by the isolative nature of the PNIPAM. Due to the stepwise decrease in side chain

density along the backbone, the redox centers have varying environments within a bottlebrush molecule, resulting in apparent multiple electron transfer processes.

7.5 Experimental section

Materials

Chemicals were purchased from Sigma-Aldrich (The Netherlands) and used as received if not stated otherwise. Organic solvents were purchased from Biosolve (Valkenswaard, The Netherlands). *N*-isopropylacrylamide (Acros Organics, Belgium) was recrystallized from hexane/toluene. Deuterated chloroform was purchased from Buchem BV (Apeldoorn, The Netherlands). CuCl and CuBr were taken up in 98% acetic acid, stirred overnight, filtered, washed with methanol and dried under vacuum. Deionized water from a MilliQ Advantage A 10 purification system (Millipore, Billerica, Ma, USA) was used. For dialysis, Spectra/Por membrane with Mw = 1000 or 15 000 cut off was used. Tris((dimethylamino)ethyl)amine (Me₆TREN) was purchased from Alfa Aesar (USA). Basic aluminium oxide 60 was purchased from Merck KGaA (Darmstadt, Germany). Vitamin-C was received from Fluka.

Methods

Nuclear Magnetic Resonance (NMR) spectroscopy

¹H NMR spectra were recorded on a Varian Unity Inova (300 MHz) at 300.3 MHz or on a Bruker Avance (400 MHz) instrument. ¹³C NMR was carried out on a Bruker Avance instrument (100 MHz). As a reference, chemical shift of the solvent signal (CDCl₃) δ = 7.26 ppm was used.

Fourier transform infrared (FTIR) spectroscopy

Single reflection attenuated total reflection (ATR) mode FTIR spectra (spectral resolution 4 cm⁻¹, 32 scans) were recorded with a Bruker model Alpha FTIR spectrometer equipped with an ATR platinum diamond 1 reflection crystal (Bruker Optik GmbH, Ettlingen, Germany). Before measurements, a background spectrum was recorded against air.

Gel Permeation Chromatography (GPC)

GPC measurements on the polymers were carried out in THF (flow rate 2.0 ml/min) at 25 °C, using microstyragel columns (bead size 10 μ m) with pore sizes of 10⁵, 10⁴, 10³, and 500 Å (Waters) with a differential refractometer (Waters model 410) detection system. Molar masses were determined relative to polystyrene standards in THF and relative to poly(methyl methacrylate) standards in DMF.

Thermogravimetric analysis

Isothermal weight loss of the polymer samples (5–10 mg) was measured under a N₂ environment (flowrate 20 ml/min) as a function of time for different temperatures with a Perkin-Elmer thermogravimetric analyzer (Pyris 1, Waltham, Ma, USA). The temperature was stepped between 50–600 ° with a rate of 10 °/min.

Electrochemistry

Electrochemical measurements were carried out on an AutoLab PGSTAT 10 electrochemical workstation. As glassy carbon was used as a working-, and Pt wire as counter electrode. In organic environment cyclic voltammograms were recorded between -0.4 and +0.6 V using 0.1 M NBu₄PF₆ in acetonitrile, with a scan rate of 50 mv/s, using Ag/Ag⁺ as a reference electrode. In aqueous solution cyclic voltammograms were recorded between 0 and +0.6 V in 0.1 M NaClO₄ solution against an Ag/AgCl reference electrode with a scan rate of 50 mv/s. Differential pulse voltammograms were measured with $v = 5$ mV/s scan rate, 50 ms pulse time, 200 ms interval time, 10 mV pulse height.

Synthesis of grPFS-Cl

In a glovebox under prepurified N₂, [1](3-chloropropyl)methylsilaferrocenophane, (0.86 g, 2.8 mmol) was dissolved in THF (13 ml), the polymerization was started by adding the catalyst, H₂PtCl₆ × 6H₂O (15 mg, 3.10⁻⁵ mol). The solution was continuously stirred and after 1, 2 and 3 hours additional monomer was introduced; [1]ethylmethylsilaferrocenophane (0.82 g, 3.2 mmol) previously dissolved in 13 ml THF, [1]ethylmethylsilaferrocenophane (0.51 g, 2.0 mmol) previously dissolved in 10 ml THF and (3-chloropropyl)methylsilaferrocenophane (0.27g, 0.9 mmol) previously dissolved in 10 ml THF, respectively. The polymerization was conducted for in total 8 hours and precipitation into MeOH afforded grPFS (0.60 g, 50%) as an orange-yellow solid. $T_g = 10$ °C.

¹H NMR (300 MHz, CDCl₃) of grPFS-Cl 0.4 ppm, (s, 3H, CH₂-Si-CH₂CH₃), 0.5 ppm, (s, 3H, CH₃-Si), 0.9 ppm (s, 2H, Si-CH₂-CH₃), 1.0 and 1.1 ppm (Si-CH₂-CH₃-CH₂ and Si-CH₂-CH₃), 1.8 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 3.5 ppm (s, 2H, Si-CH₂-CH₂-CH₂-Cl), 4.0 and 4.2 ppm (m, 4-4 H, Cp)

¹³C NMR (100 MHz, CDCl₃) of grPFS-Cl -3.3 ppm (CH₃-Si-CH₂CH₃), -3.05 ppm (CH₃-Si), 8.15 ppm (Si-CH₂-CH₃), 14.1 ppm (Si-CH₂-CH₂-), 28.0 ppm (Si-CH₂-CH₂-), 48.4 ppm (-CH₂-CH₂-N), 70.2 ppm (Cp-Si-), 70.7 ppm (Cp-Si-CH₂CH₃), 71.4 and 73.6 ppm (Cp)

ATR-FTIR of grPFS-Cl, wavenumbers (cm⁻¹) 3086 (C-H stretching vibration for ferrocene), 2931 and 2870 (asymmetric and symmetric C-H stretching vibration in -CH₂-), 1447, 1420 (C=C stretching vibration from ferrocene), 1380 (asymmetric deformation vibration of -CH₃ group on Si), 1360 and 1247 (symmetric -CH₃ deformation vibration), 1160 (asymmetric ring in-plane vibration for ferrocene), 1034 (asymmetric ring out-of-plane vibration for ferrocene), 892 (-CH₃ rocking vibration in S-CH₃), 865 (Si-C stretching vibration), 826 (in-plane C-H stretching in ferrocene), 771 and 742 (out-of-plane C-H deformation in ferrocene)

Synthesis of (grPFS-N₃)

grPFS-Cl (300 mg, 1 mmol), NaN₃ (320 mg, 5 mmol) and 15-crown-5 (460 mg, 2.06 mmol, 0.4 ml) were dissolved in a mixture of 10 ml THF and 2 ml DMSO, heated to 50 °C and stirred for 2.5 weeks under N₂ atmosphere. The product was precipitated two times into MeOH and dried in vacuum.

¹H NMR (300 MHz, CDCl₃) of grPFS-N₃ 0.4 ppm, (s, 3H, CH₃-Si-CH₂CH₃), 0.5 ppm, (s, 3H, CH₃-Si), 0.9 ppm (s, 2H, Si-CH₂-CH₃), 1.0 and 1.1 ppm (Si-CH₂-CH₃-CH₂ and Si-CH₂-CH₃) 1.6 ppm (s, 2H, Si-CH₂-CH₂-CH₂), 3.2 ppm (s, 2H, Si-CH₂-CH₂-CH₂-Cl), 4.0 and 4.2 ppm (m, 4-4 H, Cp)

ATR-FTIR of grPFS-N₃, wavenumbers (cm⁻¹) 3086 (C-H stretching vibration for ferrocene), 2931 and 2870 (asymmetric and symmetric C-H stretching vibration in -CH₂-), 2088 (azide), 1447, 1420 (C=C stretching vibration from ferrocene), 1380 (asymmetric deformation vibration of -CH₃ group on Si), 1360 and 1247 (symmetric -CH₃ deformation vibration), 1160 (asymmetric ring in-plane vibration for ferrocene), 1034 (asymmetric ring out-of-plane vibration for ferrocene), 892 (-CH₃ rocking vibration in S-CH₃), 865 (Si-C stretching vibration), 826 (in-plane C-H stretching in ferrocene), 771 and 742 (out-of-plane C-H deformation in ferrocene)

Synthesis of PNIPAM

NIPAM (6.3 g, 56 mmol), S-1-dodecyl-S'-(α,α' -dimethyl- α'' -propargyl acetate) (113 mg, 0.28 mmol), AIBN (9 mg, 0.055 mmol) and 1,4-dioxane (40 mL) were charged into a flask and purged with N₂ for one hour. The polymerization was started by heating the reaction mixture to 70 °C. The solution was stirred strongly under a small n₂ flow for 60 minutes, to obtain low molecular weight PNIPAM. The polymerization was quenched by immersing in an ethanol/N₂ bath (-50°C), and warmed up to room temperature. The solution was concentrated under vacuum, and precipitated into cold diethyl ether.

Synthesis of grBb

grPFS-N₃ (98 mg, 0.32 mmol) and **PNIPAM** (600 mg, mmol) were dissolved in a mixture of 5 ml THF and 1 ml DMSO. CuSO₄ (30 mg, 0.20 mmol) and sodium ascorbate (80 mg, 0.4 mmol) were introduced under N₂ atmosphere, then PMDETA (57 μ l, 0.4 mmol) was added. The reaction mixture was stirred for 5 days. Azide functionalized Merrifield resin³⁸ was added to remove the un reacted PNIPAM chains. The resin and the copper were removed by centrifugation, and the product was further purified by dialysis in methanol and precipitation into diethyl ether.

¹H NMR (400 MHz, CDCl₃) of grBb 0.46 ppm, (s, CH₃-Si-CH₂CH₃ and CH₃-Si), 0.9 ppm (s, Si-CH₂-CH₃), 1.1 ppm (Si-CH₂-CH₃-CH₂ and Si-CH₂-CH₂), 1.6 and 1.8 ppm (br, -CH₂-CH- (PNIPAM) and Si-CH₂-CH₂-CH₂), 2.1 ppm (br, -CH₂-CH- (PNIPAM)), 3.5 ppm (s, Si-CH₂-CH₂-CH₂-Cl), 4.0 and 4.2 ppm (m, -HC(CH₃)₂ and 4-4 H, Cp)

¹³C NMR (100 MHz, CDCl₃) of grBb , -3.3 ppm (CH₃-Si and CH₃-Si-CH₂CH₃), 7.94 and 7.88 ppm (Si-CH₂-CH₃), 14.05 ppm (Si-CH₂-CH₂-), 22.5 ppm (-C(CH₃)₂), 35.8 ppm (-NH-CH(CH₃)₂), 41.26 and 42.6 (-CH-CH₂- and CH-CH₂-), 70.5 ppm (Cp-Si-), 70.7 ppm (Cp-Si-CH₂CH₃), 71.15 and 73.36 ppm (Cp), 174.3 ppm (-C(=O)-NH-)

ATR-FTIR of grBb, wavenumbers (cm⁻¹) 3289 (N-H symmetric and asymmetric stretching vibration), 3078, 2971 and 2933 and 2874 (asymmetric and symmetric C-H stretching vibration in -CH₂-), 1635 (C=O stretching vibration), 1535 (amide II), 1458, 1386, 1366, 1170, 1037 (asymmetric ring- out-of-plane vibration for ferrocene),

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Chapter 8

Outlook: design concept of the choice of the substrate

As an outlook, we sketch the realization of a nanocrawler based on the dual stimulus responsive gradient bottlebrush that was synthesised in Chapter 7. The bottlebrush is composed of a redox responsive poly(ferrocenylsilane) (PFS) backbone and temperature responsive poly(N-isopropylacrylamide) (PNIPAM) side chains with gradually decaying grafting density along the backbone. The proposed translational movement of the nanocrawler originates from the extension-retraction of the PFS upon oxidation/reduction and the hydrophilic/hydrophobic surface property switch of the PNIPAM side chains in response to the increased temperature of the environment. To estimate the 'stickiness' of the side chains, as a model system, the affinity of a PNIPAM brush modified atomic force microscopy tip was tested on four chemically modified substrates, in atomic force microscopy based chemical force spectroscopy measurements. The obtained results show that depending on the surface energy of the various surfaces, the change in the adhesion force, upon response of the PNIPAM to the temperature increase, can be tuned. Therefore an ideal surface for a nanocrawler can be estimated in by means of surface energy.

8.1 Introduction

One may wonder about the nature of the movement of a nanocrawler we aimed at designing in this Thesis. In the previous chapters a detailed description has been provided to synthesise and characterize molecular bottlebrush molecules based on an architecture of two stimulus responsive polymers. We proposed to design a nanocrawler that would directionally move forward on the surface upon external stimuli. As a backbone a redox responsive polymer poly(ferrocenylsilane) (PFS) was used, while the side chains consist of temperature responsive poly(*N*-isopropylacrylamide) (PNIPAM). In principle during oxidation-reduction cycles the PFS periodically extends and retracts along the chain direction.¹ The response of PNIPAM to temperature manifests itself in phase separation, which corresponds to a coil-to-globule transition of the chain in aqueous solution.² As a consequence the surface properties of the collapsed, aggregated polymer change from hydrophilic to hydrophobic.³

Figure 8.1: 'Stick and slip' movement of a gradient molecular bottlebrush based nanocrawler.

Based on this knowledge, in [Figure 8.1](#) a sketch of the proposed movement is presented. In the first phase the molecule is adsorbed weakly to the underlying surface. Upon oxidation the backbone extends equally in both directions (phase 2). In phase 3 the side chains collapse in response to the change in the temperature, and adhere stronger to the surface. Due to the gradient decay of the side chains from head to tail, the strength of the attachment is inhomogeneous along the chain. Thus after reduction of the backbone in phase 4, contraction occurs unsymmetrically. By cooling the environment the PNIPAM gets detached from the surface. After completion of the first step, the nanocrawler returns to its original state.

Several criteria must be fulfilled to realize the movement of such macromolecules.^{4–6}

First of all, in order to achieve translational movement on the surface it is essential that the mechanical work subtracted from the extension-retraction of the backbone compensates for any extra adhesion originating from the interaction that may occur during the phase transition of the PNIPAM. The theoretical basis of the energy released during the cycle was established in a previous work.^{1,7} Mechanical work obtained for a single PFS chain was calculated based on the stiffness change and length extension of the polymer strand.

The other main issue of consideration is the choice of the surface. Besides that it has to be conductive, the adhesive force of the PNIPAM to the surface is critical as well. In order to obtain design criteria, we carried out AFM adhesion force measurements between a model PNIPAM modified surface and several chemically different surfaces to test its adhesive behavior.

8.2 Adhesion measurement of PNIPAM brushes on diverse surfaces at variable temperatures by AFM-CFS

To mimic the side chains of the bottlebrush molecule, PNIPAM chains were end tethered, by forming a polymer brush on an atomic force microscopy (AFM) tip.⁸ Force measurements, based on AFM (AFM-CFS), were carried out to probe interactions force between surfaces.⁹ AFM-CFS allows one to determine forces of interaction between two chemically modified surfaces, including an AFM tip and an opposing substrate. A sketch of the experimental arrangement is presented in [Figure 8.2](#). Since the polymer is attached to the AFM tip, during the measurement the contact area between the polymer and the surface remains constant.

Four different chemically modified substrates were chosen to test the affinity of the PNIPAM layers towards them. Three surfaces were decorated with different chemical groups, -OH, -CH₃, -NH₂ and the fourth one was functionalized with a (50-50%) mixture of -OH and -CH₃ headgroup bearing molecules. The functionalized surfaces were prepared by means of gold-thiol chemistry, which yielded highly ordered, homogeneous layers.¹⁰

In an aqueous solution of PNIPAM above the lower critical solution temperature (LSCT 32 °C), segment-segment and segment-solvent interactions along the polymer chain are perturbed, resulting in the precipitation of the polymer. This phase transition behavior of PNIPAM thin layers is accompanied by a hydrophilic/hydrophobic switch of their surface adhesive property.^{3,11,12}

Adhesion force measurements, below and above the LCST, were carried out to quantify this change in the adhesive properties of the PNIPAM brushes. The obtained result is represented in [Figure 8.3](#) and summarized in [Table 8.1](#).

Adhesion forces less than 50 pN were measured on the OH modified surfaces, which are the lowest among the surfaces assessed here. Furthermore, there is no variation observed with temperature.

Figure 8.2: Schematic representation of the adhesion assessment experiment. Adhesion force measurement of a PNIPAM brush grafted AFM tip on four chemically functionalized surfaces.

Figure 8.3: Adhesion forces of PNIPAM polymer brushes on different surfaces.

The highest adhesion forces were measured on the CH₃ functionalized surfaces yielding value, at room temperature of 7.35 ± 0.09 nN which shifted to 1.72 ± 0.55 nN after the temperature was raised to above T_{LCST} . On NH₂ functionalized surfaces, also relatively high adhesion force values were measured: 1.56 ± 0.45 nN at room temperature and 1.72 ± 0.55 nN at 36 °C. In both cases there is an obvious shift to higher force values upon increasing the temperature. By mixing the OH and CH₃ end functional molecules, an intermediate value was obtained, 0.56 ± 0.18

nN at low temperature, and no significant change of the value was observed above T_{LCST} , 0.54 ± 0.16 nN.

Table 8.1: Adhesion forces measured on different surfaces with PNIPAM polymer brush grafted AFM tips, below and above the LCST and the characteristic water contact angles of the substrates.

Surface	F_{adh} (nN) 27 °C	F_{adh} (nN) 36 °C	θ (°)
OH	0.05	0.05	<10
Mix	0.56 ± 0.18	0.54 ± 0.16	60 ± 4
NH₂	1.56 ± 0.45	1.72 ± 0.55	45 ± 4
CH₃	7.35 ± 0.09	7.74 ± 0.68	108 ± 2

To analyse and understand the results obtained in these measurements it is convenient to use theoretical models from contact mechanics.¹³ These models describe a relation between adhesion forces and the work of adhesion needed in order to separate two contacting bodies, *i.e.* to lift up the side chains of our nanocrawler.

Firstly, according to theory, when two contacting bodies separate, a certain amount of work must be done: a new surface area is formed that requires energy. The work is called the work of adhesion, (W_{adh}) and this depends on the various interfacial γ energies involved, in the following way:

$$W_{adh} = \gamma_{sl} + \gamma_{pl} - \gamma_{ps} \quad (8.1)$$

where the subscripts refer to the interfacial energies of the contacting phases; sl: substrate–liquid, pl: polymer–liquid and ps: polymer–substrate. Secondly, from our adhesion force measurements, when the contact area of the modified tip and the substrate is known, the work of adhesion can be calculated.¹³

To correlate the movement of the nanocrawler to these measures, its relation to them should be examined. As we propose, above T_{LCST} the nanocrawler sticks to the surface, due to the induced change of the interfacial energies. During the heating-cooling cycle, the interfacial energies of the PNIPAM water interface as well as the PNIPAM substrate interface change. Hence, any change in the work of adhesion is the result of the balance between these two terms. (We assume that the change in liquid solid interaction can be neglected.)

In conclusion, in case of our nanocrawler where mechanical work is supplied by the extension-retraction of the backbone, one can in principle calculate what the surface energy of the substrate should be in order to achieve translational movement. In addition, from water contact angle measurements (Table 8.1) of the measured surfaces, the interfacial energy between the substrate and the liquid can be determined.¹⁴ Since the **Mix** and **NH₂** modified surfaces have similar hydrophilicity but show a large difference in the measured adhesion forces, the polymer-surface inter-

facial energies are related to the chemical composition. Therefore, substrates with an appropriate chemical functionality can be chosen to achieve the desired polymer-surface interfacial energies. Corresponding research will be carried out in the future.

8.3 Experimental section

Surfaces

Thiol monolayers were formed on the substrates with varying end groups, 11-mercapto 1-undecanol, 1-dodecanthiol or cysteamine. A solution was prepared with the thiol molecules in ethanol (10^{-6} M). Gold coated substrates (100 nm gold evaporated on a <100> Si wafer having a 10 nm Cr adhesion layer) were cleaned with chloroform and piranha solutions, subsequently rinsed with Milli-Q water and ethanol and immersed for overnight in the monolayer solution. The substrates were rinsed with ethanol, dried under nitrogen flow, and immediately transferred for further measurement.

Tip modification

Immobilization of the initiator by forming SAMs on a gold coated surface

A solution was prepared with 2-bromo-2-methyl-propionic acid and 11-[11-(2-bromo-2-methyl-propionyloxy)-undecyldisulfanyl]-undecyl ester in chloroform (20 ml with concentration of 0.2 mM).¹⁵ Gold AFM probes (Bruker, Santa Barbara) and gold coated substrates (100 nm gold evaporated on Si wafer having a 10 nm Cr adhesion layer) were first cleaned with chloroform and piranha solutions, subsequently rinsed with Milli-Q water, ethanol and chloroform and immersed in the initiator solution for overnight. Gold coated flat substrates were used for further characterization by FTIR and contact angle measurement.

SI-ATRP of PNIPAM from gold coated substrates and gold colloidal probes

PNIPAM brushes were grafted from SAM modified gold substrates and AFM probes by SI-ATRP. The initiator-covered substrates were placed in dry vials and purged with argon for half an hour. The monomer *N*-isopropylacrylamide (5.6 g, 50 mmol) and PMDETA (320 μ l, 0.5 mmol) were dissolved in the ATRP medium (1.6 ml water and 18 ml methanol) and deoxygenated by bubbling with Ar for half an hour. Concurrently, CuBr (76 mg, 0.5 mmol) was added to a flask equipped with a magnetic stirrer bar and deoxygenized by purging with Ar. The degassed monomer solution was transferred into it and was stirred under Ar for a further 15 min until a clear green solution was observed. The polymerization mixture was injected into each reaction vial in a way that each sample was completely submerged. The polymerization was conducted for 0.5 hours at room temperature, and the reaction was terminated by exposing the solutions to air. The samples were subsequently removed from the vials and washed with MilliQ water and an EDTA solution to remove all the copper.

AFM measurements

A Bruker Multimode AFM with a heater-cooler setup and a NanoScope V controller was used for the variable temperature measurements, equipped with a closed quartz liquid cell as

a tip holder. The force curves were captured in MilliQ water, with a fixed ramp rate of 1 Hz and ramp size of 1 μm , therefore the forward and retract velocity was 2 $\mu\text{m/s}$. The applied load varied between 1.5 nN and 2.5 nN triggered by the deflection of the cantilever. In total 8 modified tips were used to probe the different substrates. The cantilever spring constants (0.07–0.11 N/m) were determined in liquid via the thermal tune method.¹⁶ Force distance curves were analyzed with a home written software (by Bart Kieviet) based on MatLab to obtain the adhesion force data.

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Summary

In this Thesis, addressable macromolecules based on two classes of stimulus responsive polymers, temperature responsive poly(*N*-isopropylacrylamide) (PNIPAM) and redox responsive poly(ferrocenylsilane) (PFS), are described. Our goal was to lay the foundation for a molecular crawler based on PFS-*g*-PNIPAM graft copolymers, which could exploit the dual stimulus responsiveness of these branched polymer architectures for directed motion. To this end, we established a reliable functionalization method for attaching PNIPAM as side chains to a PFS backbone, with the aid of the Huisgen cycloaddition click reaction. This allowed us to tune the structure of the formed molecular bottlebrushes, and create a grafting density gradient along the chain. The addressability of the bottlebrushes was assessed by gauging the redox response of the PFS backbone by cyclic voltammetry as a function of temperature and by studying the reversible change in molecular size by atomic force microscopy (AFM) and dynamic light scattering (DLS) measurements as a function of temperature. Furthermore, for the study of stimulus responsiveness of single chains and their ensembles, atomic force microscopy (AFM) methods were used.

A brief introduction of the topics relevant for this Thesis and a motivation for the work are presented in **Chapter 1**. Responsive polymers as well as multiresponsive materials that react reversible to external stimuli have attracted much attention over the last decades.

Chapter 2 provides an overview of stimulus responsive polymer architectures and summarizes the relevance of such materials in the area of materials science. The first part of the Chapter describes chemical methods to construct addressable macromolecules. Physical properties of such polymer structures are discussed. Furthermore, relevant tools to study the responsive behavior of these macromolecular architectures, such as dynamic light scattering and atomic force microscopy are introduced. The second part of the Chapter focuses on the application of atomic force microscopy for the investigation of polymer properties at the nanoscale.

To investigate nanomechanical properties of end grafted polymer layers (brushes) we employed AFM. In **Chapter 3**, as a representative example, a zwitterionic poly(sulfobetaine methacrylate) (PSBMA) brush, grafted from a planar Si surface and a poly(methacrylic acid) (PMAA) brush, grown on a colloidal AFM probe, were studied. Force-distance curves were obtained and the grafting density based on the theory of de Gennes was determined. The apparent value of the Young's modulus, analyzed by the Hertz model, was also determined.

AFM based single molecule force spectroscopy (AFM-SMFS) is often used for the detection and mechanical characterization of single molecules under environmentally controlled conditions. As a next step to observe single chain responsive properties, in **Chapter 4** the molecular stretching behavior of temperature responsive poly(*N*-isopropylacrylamide) (PNIPAM) chains was studied by AFM-SMFS. Force-extension

curves obtained in water below and above the lower critical solution temperature (LCST), in the co-nonsolvent mixture water/methanol, and in dimethyl sulfoxide (H-bond blocking) follow the same trajectory, regardless whether the chain was pulled from a collapsed or from a solvated state. This result indicates that for a single PNIPAM chain the formation of intrachain H-bonds in the precipitated state does not cause measurable chain stiffening at the single chain level.

For stimulus-propelled molecular crawlers we chose a system that exhibits dual responsive behavior, consisting of a PFS backbone and PNIPAM side chains (PFS-*g*-PNIPAM). In **Chapter 5** corresponding molecular bottlebrushes were discussed. These macromolecules were obtained by forming the backbone first, followed by side chain grafting. To prepare the dual stimuli responsive macromolecules a 'grafting to' as well as a 'grafting from' process were used. The 'grafting to' method involved a Huisgen cycloaddition click reaction between an azide functionalized PFS backbone and alkyne end-functionalized PNIPAM chains. According to ^1H and ^{13}C NMR spectroscopy, quantitative azido functionalization of the backbone was achieved. The click reaction between the backbone and the side chains afforded bottlebrushes with average molar masses of $M_n = 2 - 4$ million g/mol, and when clicking PNIPAM chains, approximately 75% of the azide groups were consumed, yielding bottlebrushes with relatively high grafting densities. Functionalization of the PFS backbone with smaller moieties such as pendant initiator groups via click chemistry proceeded with quantitative conversion. From one of these macroinitiators, bearing ATRP groups, PNIPAM chains were grown from the organometallic PFS macroinitiator via a 'grafting from' ARGET-ATRP process. Gel permeation chromatography measurements proved that the PFS backbone was converted into macromolecules of much higher molar mass after the 'grafting to' or 'grafting from' reactions.

Three dimensional objects such as the water-soluble graft bottlebrushes can undergo a change in their size and/or shape in response to an external environmental change. PFS-*g*-PNIPAM bottlebrushes were imaged by AFM. Height images of bottlebrush molecules deposited on a HOPG surface were obtained in air and in aqueous NaClO_4 . These images confirmed the cylindrical shape of the bottlebrush molecules. In the dry state, the average length of the molecules was 105 nm, the width was 15 nm and the height 5 nm. In liquid environment the bottle brush molecules retained their shape, but they swelled about 3 - 5 times their original size in width and height. The length of the molecules did not change significantly. The dimensions of the bottlebrushes obtained by AFM matched well with those obtained by dynamic light scattering. The response to temperature of the PNIPAM side chains, and variation in the oxidation state of ferrocene in the backbone were studied in detail as a function of the structure of the bottlebrushes in **Chapter 6**. For all PFS-*g*-PNIPAM bottlebrushes, in aqueous solution, LCST behavior was observed, and T_{LCST} was determined to be 32 °C, which is identical to that of PNIPAM. As monitored by dynamic light scattering measurements bottlebrushes undergo a reversible linear decrease in size over a wide temperature range, until the LCST is reached where either intra- or intermolecular collapse was observed. PFS-*g*-PNIPAM macromolecules deposited

on a HOPG surface showed two different types of responses to electrochemical signals. In most of the cases a double-wave cyclic voltammogram typical of PFS was observed. The shape of the voltammogram changed when the temperature was increased above the LCST, indicating that the redox response is influenced by the temperature response of PNIPAM. In contrast to these measurements, bottlebrush with long PNIPAM side chains displayed 'break in' behaviour upon oxidation at 22 °C, and only one oxidation peak was observed at 0.5 V.

A bottlebrush with a gradient in side chain grafting density was obtained by creating a composition gradient within the PFS backbone consisting of functionalizable monomer units and nonfunctionalizable ones. **Chapter 7** describes the synthesis of the asymmetrical PFS-g-PNIPAM bottlebrushes. By controlling the feed ratio of the two [1]silaferrocenophane monomers, featuring ethyl-methyl or methyl-chloropropyl groups at the silicon atom, a continuous composition decay of the latter units was achieved. After backbone formation, the chloropropyl moieties were converted into azidopropyl groups, and according to ^1H NMR measurements the final product contained 35% azide groups and 65% of inert ethyl-methyl substituted units. The obtained monomodal peak in gel permeation chromatography traces and the steadily increasing molar mass with time confirmed that copolymerization of the monomers occurred. Alkyne end-functionalized PNIPAM chains were attached in a 'grafting to' process via click reaction. Based on ^1H NMR- and ATR-FTIR spectroscopy we estimate that the click reaction between the backbone and the side chains afforded a gradient bottlebrush with a molar mass of $M_n = 2.7$ million g/mol. Dual stimuli responsive properties of the gradient bottlebrush were assessed by cyclic voltammetry (CV) measurements. In organic electrolyte, reversible double wave oxidation curves were obtained, while in aqueous environment the shape of the voltammograms was highly influenced by the inhomogeneous distribution of the water soluble PNIPAM chains along the backbone and further changed when the temperature was raised above the LCST.

In principle, during oxidation-reduction cycles, the PFS chain periodically extends and retracts along the chain direction, while the phase transition of PNIPAM manifests itself in an adhesion change towards the underlying surface. Therefore, a sequential variation in the externally applied stimuli should result in directed motion of the molecule on a suitable surface. In **Chapter 8** we summarize principles of the molecular movement of a nanocrawler and discuss how characteristics of the underlying surface can be in principle tuned to assist in directional molecular motion.

Samenvatting

In dit Proefschrift worden aanstuurbare macromoleculen, gebaseerd op twee typen 'stimuli-responsive' polymeren, temperatuur responsive poly(*N*-isopropylacrylamide) (PNIPAM) en redox responsive poly(ferrocenylsilaan) (PFS) beschreven. Ons doel was om de basis te leggen voor een moleculaire 'crawler' gebaseerd op PFS-*g*-PNIPAM graft copolymeren, die de dubbele stimulus responsivens van deze vertakte polymeerarchitecturen kunnen gebruiken voor beweging in een gewenste richting. Hiertoe werd een betrouwbare functionaliseringsmethode ontwikkeld voor het hechten van PNIPAM zijketens aan een PFS hoofdketen, door middel van de Huisgen cycloaditie 'click' reactie. Door middel van deze methode kon de structuur van de gevormde moleculaire bottlebrushes worden beïnvloed en kon een gradient in de dichtheid van zijketens worden gerealiseerd. De reactie van de bottlebrush moleculen op stimuli werd onderzocht door de redox respons van de PFS hoofdketen te bepalen met behulp van cyclische voltammetrie als functie van temperatuur en door het bestuderen van de reversibele verandering in moleculaire grootte door atomic force microscopy (AFM) en dynamische lichtverstrooiingsmetingen (DLS) als functie van temperatuur. Bovendien werden voor het bestuderen van de stimuli responsivens van enkelvoudige ketens en hun ensembles atomic force microscopy methoden gebruikt.

Een korte inleiding van de onderwerpen die relevant zijn voor dit Proefschrift en een motivatie voor het werk worden gepresenteerd in **Hoofdstuk 1**. De belangstelling voor op stimuli reagerende polymeren en materialen die reageren op meerdere typen stimuli is in de afgelopen tientallen jaren voortdurend gestegen. **Hoofdstuk 2** geeft een overzicht van op stimuli reagerende polymeerarchitecturen en vat de relevantie van zulke materialen op het gebied van materiaalkunde samen. Het eerste deel van het Hoofdstuk beschrijft chemische methoden om materialen te maken die kunnen reageren op externe stimuli. Fysische eigenschappen van dergelijke polymeerstructuren worden besproken. Bovendien worden relevante technieken om het responsieve gedrag van deze macromoleculaire architecturen te bestuderen, zoals dynamische lichtverstrooiing en atomic force microscopy geïntroduceerd. Het tweede deel van dit Hoofdstuk richt zich op de toepassing van atomic force microscopy voor het onderzoeken van polymeereigenschappen op nanoschaal. Om nanomechanische eigenschappen van eind-geïmmobiliseerde polymeerlagen (brushes) te onderzoeken werd AFM gebruikt. In **Hoofdstuk 3** worden, als een representatief voorbeeld, een zwitterionische poly(sulfobetaine methacrylaat) (PSBMA) brush, gegroeid vanaf een vlak Si oppervlak en een poly(methacrylzuur) (PMAA) brush, gegroeid vanaf een colloïdale AFM probe, bestudeerd. Kracht-afstandscurves werden verkregen en de verankeringsdichtheid van de polymeerketens, gebaseerd op de theorie van de Gennes, werd bepaald. De schijnbare waarde van de Young's modulus, geanalyseerd door middel van het Hertz model, werd ook bepaald. Op AFM gebaseerde single molecule force spectroscopy (AFM-SMFS) wordt vaak gebruikt voor de detectie en mechanische

karacterisering van enkelvoudige moleculen onder gecontroleerde omstandigheden. Als volgende stap om de responsieve eigenschappen van enkelvoudige ketens te onderzoeken, behandelt **Hoofdstuk 4** de studie van het moleculaire verstrekkingsgedrag van op temperatuurverandering reagerende poly(*N*-isopropylacrylamide) ketens door AFM-SMFS. Kracht-extensiecurves gemeten in water, onder en boven de lower critical solution temperature (LCST), in het co-nonsolvent mengsel water/methanol, en in dimethylsulfoxide (H-bond blokkerend) vertonen hetzelfde verloop, ongeacht of de keten werd verstrekt uit een samengetrokken of uit een gesolvateerde toestand. Dit resultaat geeft aan dat de vorming van intramoleculaire H-bruggen in de geprecipiteerde toestand voor een enkelvoudige PNIPAM keten geen meetbare ketenverstijving geeft op het niveau van enkelvoudige ketens. Voor moleculaire crawlers voortbewogen door stimuli kozen we een systeem dat een dubbel responsief gedrag vertoont, bestaand uit een PFS hoofdketen en PNIPAM zijketens (PFS-*g*-PNIPAM). In **Hoofdstuk 5** werden dergelijke moleculaire bottlebrushes verkregen door de hoofdketen te vormen, gevolgd door aanhechting van de zijketens. Om de dubbel responsieve macromoleculen te fabriceren werden een 'grafting to' en een 'grafting from' proces gebruikt. De 'grafting to' methode omvatte een Huisgen cycloadditie click reactie tussen een azide-gefunctionaliseerde PFS hoofdketen en alkyn eindgefunctionaliseerde PNIPAM ketens. Volgens ^1H en ^{13}C NMR spectroscopie werd een volledige azide functionalisering van de hoofdketen bereikt. De click reactie tussen de hoofdketen en de zijketens gaf bottlebrushes met gemiddelde molecuulmassa's van $M_n = 2\text{--}4$ miljoen g/mol, en bij het clicken van de PNIPAM ketens werd ongeveer 75% van de azide groepen verbruikt, wat leidde tot bottlebrushes met een relatief hoge dichtheid van aangehechte zijketens. Functionalisering van de PFS hoofdketen met kleinere groepen zoals initiator groepen door middel van click chemie verliep met een volledige omzetting. Vanaf een van deze macroinitiators, bezet met ATRP groepen, werden PNIPAM ketens gegroeid vanaf de PFS organometaal macroinitiator via een 'grafting from' ARGET-ATRP proces. Gel permeatiechromatografie metingen toonden aan dat de PFS hoofdketens waren omgezet in macromoleculen met een veel hogere molecuulmassa na de 'grafting to' of 'grafting from' reacties. Driedimensionale objecten zoals de wateroplosbare bottlebrushes kunnen een verandering in hun grootte en/of vorm ondergaan in reactie op een verandering in hun omgeving. PFS-*g*-PNIPAM bottlebrushes werden gevisualiseerd door middel van AFM. Hoogte afbeeldingen van Bb2 moleculen, aangebracht op een HOPG oppervlak, werden gemaakt in lucht en in een oplossing van NaClO_4 in water. Deze afbeeldingen toonden aan dat de bottlebrush moleculen een cilindrische vorm bezaten. In droge toestand bedroeg de gemiddelde lengte van de moleculen 105 nm, de breedte 15 nm en de hoogte 5 nm. In een vloeistofomgeving behielden de bottlebrush moleculen hun vorm, maar zwollen tot ongeveer 3-5 maal hun oorspronkelijke grootte in breedte en hoogte. De lengte van de moleculen veranderde niet noemenswaardig. De afmetingen van de bottlebrush moleculen gemeten door AFM kwamen goed overeen met de waarden verkregen door dynamische lichtverstrooiingsmetingen. De reactie van de PNIPAM zijketens op temperatuurverandering en de verandering van de oxidati-

etoestand van ferrocen eenheden in de hoofdketen werden in detail bestudeerd als functie van de bottlebrush structuur in **Hoofdstuk 6**. In waterige oplossing werd voor alle PFS-*g*-PNIPAM bottlebrush moleculen een LCST gedrag waargenomen, en een T_{LCST} bepaald van 32 °C, welke identiek is aan die van PNIPAM. Zoals bleek uit dynamische lichtverstrooiingsmetingen, ondergaan bottlebrush moleculen een reversibele lineaire afname in grootte over een breed temperatuurgebied, totdat de LCST wordt bereikt, waar intra- of intermoleculaire aggregatie optreedt. PFS-*g*-PNIPAM macromoleculen aangebracht op een HOPG oppervlak vertoonden twee verschillende typen reacties op electrochemische signalen. In de meeste gevallen werd een voor PFS kenmerkend double-wave cyclisch voltammogram waargenomen. De vorm van het voltammogram veranderde wanneer de temperatuur werd verhoogd tot boven de LCST, wat aantoont dat de redox respons wordt beïnvloed door de thermische respons van PNIPAM. In tegenstelling tot deze metingen vertoonde een bottlebrush met lange PNIPAM zijketens een 'break in' gedrag bij oxidatie bij 22 °C, en slechts een enkele oxidatiepiek bij 0.5 V. Een bottlebrush molecuul met een gradient in de aanhechtingsdichtheid van zijketens werd verkregen door een PFS hoofdketen te synthetiseren uit functionaliseerbare en niet-functionaliseerbare monomeren, met een gradient in samenstelling. **Hoofdstuk 7** beschrijft de synthese van de asymmetrische PFS-*g*-PNIPAM bottlebrush moleculen. Door het sturen van de toevoerverhouding van de twee [1]silaferrocenophane monomeren, bestaand uit ethyl-methyl of methyl-chloropropyl groepen op het silicium atoom werd een continu verloop in de verhouding van de twee repeterende eenheden bereikt. Na vorming van de hoofdketen werden de chloropropyl eenheden omgezet in azidopropyl groepen, en volgens ^1H NMR metingen bevatte het eindproduct 35% azide groepen en 65% inerte ethyl-methyl gesubstitueerde eenheden. De gemeten monomodale piek in het gel permeatiechromatogram en de, in de tijd geleidelijk toenemende molecuulmassa bevestigden dat copolymerisatie van de monomeren plaatsvond. Alkyn-eindgefunctionaliseerde PNIPAM ketens werden aan de PFS hoofdketen gehecht in een 'grafting to' proces via een click reactie. Op basis van ^1H NMR en ATR-FTIR spectroscopie schatten we dat de click reactie tussen de hoofdketen en zijketens een gradient bottlebrush opleverde met een molecuulmassa van $M_n = 2.7$ miljoen g/mol. Meervoudige stimuli responsieve eigenschappen van de gradient bottlebrush werden bestudeerd door cyclische voltammetriemetingen. In organische electrolytoplossingen werden reversibele double-wave oxidatiecurves waargenomen, terwijl in waterige media de vorm van de voltammogrammen sterk werd beïnvloed door de inhomogene verdeling van de wateroplosbare PNIPAM zijketens langs de hoofdketen, en verder veranderde wanneer de temperatuur werd verhoogd tot boven de LCST. In principe strekt zich en krimpt de PFS hoofdketen periodiek in de ketenrichting tijdens oxidatie en reductiecycli, terwijl de faseovergang van PNIPAM zich manifesteert als een verandering in adhesie ten opzichte van het onderliggende oppervlak. Daarom zou een sequentiële verandering in de extern toegevoerde stimuli moeten leiden tot een gerichte beweging van het molecuul op een oppervlak. In **Hoofdstuk 8** worden de principes van moleculaire beweging van een nanocrawler samengevat en wordt bediscussieerd hoe eigenschappen

van het onderliggende oppervlak kunnen worden beïnvloed om moleculaire beweging te ondersteunen.

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About the author

Edit Kutnyánszky was born on July 25, 1984 in Dombóvár, Hungary. After graduating from secondary school (Illyés Gyula Gimnázium, Dombóvár) she studied chemistry at the Eötvös Loránd University in Hungary. She joined the Laboratory of Interfaces and Nanosize Systems group under the supervision of Prof. Dr. Éva Kiss in 2006, where she conducted her master research, focusing on characterization and modification of biocompatible PLGA polymer surfaces. She obtained her M.Sc. degree in June 2008. In September 2008 she started her PhD project in the MTP group, headed by Prof. Dr. G. Julius Vancso, at the University of Twente, The Netherlands, focusing on stimulus responsive polymeric systems, synthesis of dual stimulus responsive copolymers and the evaluation of responses at the single chain level by atomic force microscopy and electrochemical techniques. The results of the four year research are presented in this Thesis.