PARTICLES IN POLYMER-FUNCTIONALIZED MICROCHANNELS

electrochemical experiments and molecular dynamics simulations

B.D. KIEVIET

Particles in polymer-functionalized microchannels:

Electrochemical experiments and molecular
dynamics simulations

B. D. Kieviet

Members of the committee:

Chair:	prof. dr. ir. J.W.M. Hilgenkamp	University of Twente
Promotor:	prof. dr. G.J. Vancso	University of Twente
Assistant-promotor:	dr. P.M. Schön	University of Twente
Members:	prof. dr. D. Anselmetti	University of Bielefeld
	dr. O. Prucker	University of Freiburg
	prof. dr. S. Luding	University of Twente
	prof. dr. J.C.T. Eijkel	University of Twente
	dr. S.J.A. de Beer	FOM

This research is supported by the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO) and partly funded by the Ministry of Economic Affairs (project number 11499) and by the MESA+ Institute for Nanotechnology of the University of Twente.

The work described in this thesis was carried out at the Materials Science and Technology of Polymers (MTP) group, MESA+ Institute for Nanotechnology, Faculty of Science and Technology, University of Twente, the Netherlands.

ISBN: 978-90-365-4076-6

DOI: 10.3990/1.9789036540766

PARTICLES IN POLYMER- FUNCTIONALIZED MICROCHANNELS: ELECTROCHEMICAL EXPERIMENTS AND MOLECULAR DYNAMICS SIMULATIONS

PROEFSCHRIFT

ter verkrijging van
de graad van doctor aan de Universiteit Twente,
op gezag van de rector magnificus
prof. dr. H. Brinksmā,
volgens besluit van het College voor Promoties,
in het openbaar te verdedigen
op vrijdag 29 april 2016 om 14:45 uur

door

Bernard Dingeman Kieviet

geboren op
11 april 1984 te Utrecht

Dit proefschrift is goedgekeurd door:

Promotor:	prof. dr. G.J. Vancso
Assistant-promotor:	dr. P.M. Schön

Soli Deo Gloria

Chapter 1	General introduction	9
1.1	Introduction.....	9
1.2	Content of the thesis.....	10
1.3	References	11
Chapter 2	Electrochemical detection and molecular dynamics simulations	13
2.1	Introduction.....	13
2.2	Electrochemical methods	13
2.2.1	Cyclic voltammetry.....	13
2.2.2	Chronoamperometry	16
2.2.3	Ultramicroelectrodes	17
2.3	Electrochemistry in microfluidic devices.....	18
2.3.1	Fabrication of in-channel electrodes	18
2.3.2	In-chip electrochemical sensing of (bio)molecules	23
2.4	Polymer brushes.....	26
2.5	Molecular dynamics simulations.....	27
2.5.1	Basics of molecular dynamics simulations.....	27
2.5.2	Molecular dynamics simulations on polymer brushes.....	29
2.6	References	36
Chapter 3	Stimulus-responsive polymers and other functional polymer surfaces as components in glass microfluidic channels	41
3.1	Introduction.....	42
3.2	pH-responsive polymers in fluidics.....	45
3.3	Photoresponsive components.....	46
3.4	Thermoresponsive constituents	46
3.5	Passive elements	50
3.6	Conclusions	58
3.7	References	59
Chapter 4	In-line sensing of sodium ascorbate using a poly(ferrocenylsilane)-coated microfluidic device	65
4.1	Introduction.....	66
4.2	Device fabrication.....	67
4.2.2	Gold-coated microchannels	67
4.2.2	Surface attachment of poly(ferrocenylsilane).....	69
4.3	Vitamin C sensing	71

4.3.1. Static sensing.....	71
4.3.2. In-line vitamin C sensing.....	72
4.4 Conclusions.....	75
4.5 Experimental section.....	75
4.6 References.....	76
Chapter 5 Geometry-dependent insertion-forces on particles in swollen polymer brushes	81
5.1 Introduction.....	82
5.2 Model and methods.....	84
5.2.1 Molecular Dynamics simulations.....	84
5.2.2 Theoretical model.....	86
5.3 Results and discussion.....	90
5.4 Conclusions.....	96
5.5 References.....	97
Chapter 6 Optimal shaking conditions for particle-absorption in swollen polymer brushes	101
6.1 Introduction.....	102
6.2 Model and methods.....	103
6.3 Results and discussion.....	106
6.3.1 Shaking brushes without particles.....	106
6.3.2 Static measurements with particles.....	108
6.3.3 Oscillatory measurements with particles.....	109
6.4 Conclusions.....	115
6.5 References.....	115
Chapter 7 Outlook	119
7.1 Sorption of particles on PNIPAM covered microchannels.....	119
7.2 Towards redox-responsive brushes in microchannels.....	121
7.3 Experimental section.....	121
7.4 References.....	122
Appendix.....	123
Summary.....	139
Samenvatting.....	141
Acknowledgements.....	143
About the author.....	147

General introduction

1.1 Introduction

Microfluidics has been a powerful tool in fast, reliable and low-cost detection of (macro)molecules. This should not come as a surprise, since the origins of microfluidics lie (among others) within gas-phase chromatography, high-pressure liquid chromatography and capillary electrophoresis.^[1] Miniaturization of these techniques resulted in an increase in sensitivity due to the increase in surface/volume ratio, as well as the capability to analyze small amounts of sample within relatively short times.

The success of the primal microfluidic devices has led to the development and improvement of in-chip liquid-control,^[2] such as valves^[3] and pumps^[4] operated by pressure,^[5] pH,^[6] electric field,^[7] magnetic field,^[8] temperature^[9] or light.^[10] The fact that the flow inside a micro-sized channel is laminar,^[11] stimulated the development of micromixers,^[12] and via changes in channel architecture, to flow-focusing devices.^[13] The latter resulted in the fabrication of micro-droplet generators, where droplets of one fluid phase are suspended in a second fluid phase.^[14-15] These micro-droplet generators can be employed in various synthetic pathways.^[16-17] Furthermore, microfluidics is used frequently in biological analysis^[18] and development of pharmaceuticals.^[19]

The integration of novel channel-linings has greatly improved the performance of these devices. For example, polymer-coated microchannels are used for control of electro-osmotic mobility,^[20-21] for the reduction of biofouling^[22-23] or as enzymatic microreactors.^[24-25] Furthermore, polymer coatings are used to increase the sensitivity of microfluidic sensors.^[26-27] However, so far, redox-responsive polymer-coatings have found little use in microfluidic applications. In this thesis, a redox-responsive poly(ferrocenylsilane) (PFS) is employed as a redox-active electron-mediator for electrochemical detection of sodium ascorbate via electrocatalysis.

In these polymer-coated microfluidic devices, particles are detected and identified. However, the precise interaction between the polymers and the particles on the molecular scale is not fully understood. Therefore, molecular dynamics simulations are employed to improve the understanding of the change in inclusion force when the size, shape and orientation of particles that are inserted in a polymer brush are varied. Furthermore, the effect of oscillating the substrate on the distribution of particles within the polymer brush is investigated, since most polymer-particle systems, such as the small intestine, are in some kind of motion. Both the electrochemical experiments and the

molecular dynamics simulations encompass polymer systems that are out of equilibrium and give insight in particle-polymer interaction.

1.2 Content of the thesis

Chapter 2 comprises a short review of the electrochemical methods employed in this thesis, as well as the current state of the literature concerning both fabrication and utilization of integrated electrodes in microchannels. Furthermore, a concise overview of the basics of molecular dynamics simulations and of simulations on polymer-brush systems is given.

In chapter 3, the use of polymer coatings in microfluidic devices as both active and passive layers is reviewed. The focus of the review is on microfluidic devices fabricated from glass. In this chapter, the versatility of polymer-coated microchannels becomes clear.

Chapter 4 describes various electrochemical experiments performed on a microfluidic device with integrated gold electrodes. The device was first characterized by detection of potassium hexacyanoferrate in aqueous solution. Subsequently, the electrodes were coated with a layer of PFS via amine alkylation. As a test case, the device was used for sensing of sodium ascorbate, or Vitamin C, in both static and flowing liquid and a detection limit of 0.5 mM was obtained.

In chapter 5, molecular dynamics simulations were performed on particles of various shapes and sizes, inserted into a polymer brush. The explored shapes were spheres, cubes, discs and rods, which were inserted at various angles with the brush-covered surface. The forces on the particles were modelled using two different pathways, one via scaling arguments and one aided through molecular dynamics simulations.

Chapter 6 shows a follow-up molecular dynamics simulation of a polymer brush and particle system, where the brush-covered surface is set to oscillate. The penetration depth of the spherical particles into the brush was determined via the brush and particle densities. Particles of various sizes were used, resulting in a size, an amplitude and a period dependency of the penetration depth of the particles.

Finally, chapter 7 gives an outlook towards future research on both PFS-functionalized and brush-covered microchannels.

1.3 References

- [1] G. M. Whitesides, *Nature* **2006**, *442*, 368-73 | 10.1038/nature05058
- [2] D. R. Reyes, D. Iossifidis, P.-A. Auroux, A. Manz, *Anal. Chem.* **2002**, *74* (12), 2623-2636 | 10.1021/ac0202435
- [3] J. M. K. Ng, I. Gitlin, A. D. Stroock, G. M. Whitesides, *Electrophoresis* **2002**, *23* (20), 3461-3473 | 10.1002/1522-2683(200210)23:20<3461::aid-elps3461>3.0.co;2-8
- [4] P. Woias, *Sensors Actuators B: Chem.* **2005**, *105*, 28-38 | 10.1016/j.snb.2004.02.033
- [5] W. H. Grover, A. M. Skelley, C. N. Liu, E. T. Lagally, R. A. Mathies, *Sensors Actuators B: Chem.* **2003**, *89*, 315-323 | 10.1016/S0925-4005(02)00468-9
- [6] D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss, B.-h. Jo, *Nature* **2000**, *404*, 588-590 | 10.1038/35007047
- [7] W. Satoh, M. Loughran, H. Suzuki, *J. Appl. Phys.* **2004**, *96* (1), 835 | 10.1063/1.1739528
- [8] S. Böhm, W. Olthuis, P. Bergveld, *Sensors and Actuators A: Physical* **1999**, *77* (3), 223-228 | 10.1016/S0924-4247(99)00192-2
- [9] W. L. Benard, H. Kahn, A. H. Heuer, M. A. Huff, *Journal of Microelectromechanical Systems* **1998**, *7* (2), 245-251 | 10.1109/84.679390
- [10] S. Sugiura, K. Sumaru, K. Ohi, K. Hiroki, T. Takagi, T. Kanamori, *Sensors and Actuators A: Physical* **2007**, *140*, 176-184 | 10.1016/j.sna.2007.06.024
- [11] T. M. Squires, S. R. Quake, *Reviews of Modern Physics* **2005**, *77* (3), 977-1026 | 10.1103/RevModPhys.77.977
- [12] C.-Y. Lee, W.-T. Wang, C.-C. Liu, L.-M. Fu, *Chem. Eng. J.* **2016**, *288*, 146-160 | 10.1016/j.cej.2015.10.122
- [13] S. L. Anna, N. Bontoux, H. A. Stone, *Appl. Phys. Lett.* **2003**, *82* (3), 364 | 10.1063/1.1537519
- [14] G. F. Christopher, S. L. Anna, *J. Phys. D: Appl. Phys.* **2007**, *40* (19), R319-R336 | 10.1088/0022-3727/40/19/r01
- [15] S. Y. Teh, R. Lin, L. H. Hung, A. P. Lee, *Lab on a chip* **2008**, *8* (2), 198-220 | 10.1039/b715524g
- [16] H. Song, D. L. Chen, R. F. Ismagilov, *Angew. Chem. Int. Ed. Engl.* **2006**, *45* (44), 7336-56 | 10.1002/anie.200601554
- [17] X. Sui, L. Shui, J. Cui, Y. Xie, J. Song, A. van den Berg, M. A. Hempenius, G. J. Vancso, *Chem. Commun.* **2014**, *50* (23), 3058-60 | 10.1039/c3cc49501a
- [18] S. K. Sia, G. M. Whitesides, *Electrophoresis* **2003**, *24* (21), 3563-76 | 10.1002/elps.200305584
- [19] J. El-Ali, P. K. Sorger, K. F. Jensen, *Nature* **2006**, *442* (7101), 403-11 | 10.1038/nature05063
- [20] S. Hu, X. Ren, M. Bachman, C. E. Sims, G. P. Li, N. L. Allbritton, *Langmuir* **2004**, *20* (13), 5569-5574 | 10.1021/la049974l
- [21] S. L. Barker, D. Ross, M. J. Tarlov, M. Gaitan, L. E. Locascio, *Anal. Chem.* **2000**, *72*, 5925-9 | 10.1021/ac0008690
- [22] X. Sun, J. Liu, M. L. Lee, *Electrophoresis* **2008**, *29* (13), 2760-7 | 10.1002/elps.200800005
- [23] H. Schmolke, S. Demming, A. Edlich, V. Magdanz, S. Buttgenbach, E. Franco-Lara, R. Krull, C. P. Klages, *Biomicrofluidics* **2010**, *4* (4), 44113 | 10.1063/1.3523059
- [24] A. G. Papay, *Lubr. Eng.* **1983**, *39*, 419-426 |

- [25] F. Costantini, E. M. Benetti, D. N. Reinhoudt, J. Huskens, G. J. Vancso, W. Verboom, *Lab on a chip* **2010**, *10*, 3407-12 | 10.1039/c0lc00187b
- [26] D. Belder, A. Deege, F. Kohler, M. Ludwig, *Electrophoresis* **2002**, *23* (20), 3567-3573 | 10.1002/1522-2683(200210)23:20<3567::aid-elps3567>3.0.co;2-3
- [27] Q. L. Zhang, J. J. Xu, H. Z. Lian, X. Y. Li, H. Y. Chen, *Anal. Bioanal. Chem.* **2007**, *387* (8), 2699-704 | 10.1007/s00216-007-1173-7

Electrochemical detection and molecular dynamics simulations

2.1 Introduction

In this chapter, both the electrochemical methods as the molecular dynamics (MD) simulations employed to perform experiments on particles in polymer-covered microchannels are discussed. Furthermore, a selected overview is given of the literature available on fabrication and application of in-channel electrodes, and MD simulations on polymer brush systems.

2.2 Electrochemical methods

In chemistry, electrochemistry encompasses the study of the combination of electrical and chemical effects.^[1] Mostly, this contains the transfer of electrons from or to ions or molecules via electrical means or via a chemical reaction. These electrons are often measured in a so-called three electrode configuration: the current is measured from a working electrode (WE) to a counter electrode (CE). Via a third electrode, the reference or auxiliary electrode (RE), the potential over the RE and the WE is controlled. The study of these redox phenomena has found its use in a wide variety of fields. By employing electrochemical methods, a huge collection of analytes has been sensed and studied,^[2-3] for example, vitamin C^[4] or glucose.^[5] In reactions, the electrocatalytic effect is used substantially.^[6-8] In biology, the redox characteristics of enzymes^[9-11] and membrane functions^[12-13] are of particular interest for electrochemical studies. Furthermore, electrochemistry has played a substantial role in the development of fuel cells,^[14-16] and the improvement of these fuel cells by using electroactive materials.^[17-20] In this thesis, various electrochemical methods are employed, such as cyclic voltammetry. These are discussed briefly in the following sections.

2.2.1 Cyclic voltammetry

Cyclic voltammetry is one of the more widely used electrochemical techniques. To perform a cyclic voltammetry measurement, the potential is ramped from a certain potential E_{init} linearly to a certain potential E_{end} and back, usually below and above the redox potential of the species one wants to investigate. By recording the current, information can be obtained about the redox potential, the diffusion constant, the size of the electrode, etc. A typical voltammogram of a reversible redox species in solution is depicted in Figure 2.1.

Figure 2.1: (a) A typical voltammogram of a reversible redox species in solution. Simulated using Ref. [21] Parameters used are: $n = 1$, $A = 0.5 \text{ cm}^2$, $E^0 = 0 \text{ V}$, $C = 1 \text{ mol m}^{-3}$, $v = 100 \text{ mV s}^{-1}$. (b) Potential as function of time.

As mentioned above, from Figure 2.1 several characteristics of the system can be determined by using the Randles-Ševčík equation^[22-23] (at room temperature):

$$i_p = 2.69 \cdot 10^5 n^{3/2} AC \sqrt{Dv}, \quad (2.1)$$

with i_p the height of the peak, n the number of electrons transferred in a single electrochemical reaction, A the area of the electrode, C the concentration of the electroactive species, D the diffusion constant of the electroactive species and v the scan rate. (Symbols and their explanation can be found in Table 2.1, at the end of this chapter, if descriptions are omitted). If the area of the electrode and the concentration are known, one can find the diffusion coefficient through the peak height. Or, if the diffusion coefficient is known, one can characterize the area of the electrode, for example.

From the shape of the voltammogram a lot of information can be deduced as well. For example, in a quasireversible system, the redox reaction is dependent on a rate constant, k . The effect of the rate constant on the shape of the voltammogram can be seen in Figure 2.2.

Figure 2.2: The effect of the rate constant on the voltammogram of a quasireversible solution species. a) Cyclic voltammograms of rate constants 10^{-2} m s^{-1} to 10^{-6} m s^{-1} . b) Normalized (to 57 mV) peak difference (black, left axis) and normalized peak height of oxidation (blue, right axis) and reduction (red, right axis) wave. Parameters used are: $n = 1$, $A = 0.5 \text{ cm}^2$, $E^0 = 0 \text{ V}$, $C = 1 \text{ mol m}^{-3}$, $v = 0.1 \text{ V s}^{-1}$, $D_O = 10^{-9} \text{ m}^2 \text{ s}^{-1}$.

As can be seen in Figure 2.2, from $k = 10^{-4} \text{ m s}^{-1}$ and lower, the rate constant has a tremendous influence on the shape of the wave, the peak height and peak separation. Also, varying the scan rate instead of k also has an effect on peak separation and peak height, hence one should take care when probing one when varying the other.

The above relations are valid for redox species in solution. If a species is surface-confined, the current is given by:

$$i = \frac{n^2 F^2}{RT} \frac{v A \Gamma_O^* \left(\frac{b_O}{b_R} \right) \exp \left(\left(\frac{nF}{RT} \right) (E - E^0) \right)}{\left[1 + \left(\frac{b_O}{b_R} \right) \exp \left(\left(\frac{nF}{RT} \right) (E - E^0) \right) \right]^2} \quad (2.2)$$

and the peak height is calculated as the maximum of Equation 2.2, which is:

$$i_p = \frac{n^2 F^2}{4RT} v A \Gamma_O^*. \quad (2.3)$$

From this can be concluded that the peak height of a species that is adsorbed to the surface has a linear relation with the scan rate, compared to $v^{1/2}$ in case of a diffusion controlled redox species. This is due to the assumption that the oxidation (or reduction) of the species occurs so fast, it cannot diffuse away from the electrode, or the solution species is not electroactive. A voltammogram for an ideal Nernstian reaction of a surface confined species is shown in Figure 2.3.

Figure 2.3: Normalized cyclic voltammogram of a surface confined redox species. Copyright 2001 © John Wiley & Sons, Inc. All rights reserved.^[1]

From the area below the peak, one can obtain the total charge required to fully oxidize or reduce the layer:

$$Q = \int i \, dt = v^{-1} \int i \, dE = nFA\Gamma_0^*. \quad (2.4)$$

Especially for surface confined redox species, knowing the surface coverage is paramount, as it gives an indication if the quality of the layer formed. For example, for ferrocene-based monolayers this is $0.77 \, \text{nmol cm}^{-2}$.^[24]

2.2.2 Chronoamperometry

Another technique employed in this thesis is chronoamperometry. When performing chronoamperometry experiments, instead of ramping the potential as is done in cyclic voltammetry, the potential is set at a certain value E , and subsequently the current response is measured over time. This response is governed by the Cottrell equation:^[25]

$$i = nFAD_0^{1/2} C_0^* (\pi t)^{-1/2}. \quad (2.5)$$

A typical result of a chronoamperometry experiment is shown in Figure 2.4.

Figure 2.4: Typical chronoamperometric response of a redox species in solution. Parameters used are: $n = 1$, $D_O = 10^{-9} \text{ m}^2 \text{ s}^{-1}$, $C_O = 1 \text{ mol m}^{-3}$, $A = 0.5 \text{ cm}^2$.

When comparing chronoamperometry with cyclic voltammetry one can conclude that the latter provides more qualitative information about the system studied than the former. However, by using chronoamperometry, solution concentrations can be more accurately determined. More detail on these techniques can be found in Péter^[26] and Bard and Faulkner.^[1]

2.2.3 Ultramicroelectrodes

When the dimensions of the measuring electrodes are made significantly smaller, electrical field-effects that are insignificant in macro-sized electrodes are becoming of influence. These ultramicroelectrodes (UMEs) have at least one dimension in the micrometer range. This small size leads to low currents, however, both the ohmic drop (IR) and the electrode time constants (RC) are reduced.^[27] Because mass transport is faster at smaller electrodes, a steady state current can be reached, depending on UME geometry. This steady-state current is given by:

$$i_{ss} = nFADC \cdot m_O, \quad (2.6)$$

where m_O is a mass-transfer coefficient depending on the geometry. For a hemisphere, $m_O = r_0^{-1}$, for example.^[1] This steady-state current is reached at the time scale where the diffusion layer is a lot larger than r_0 , otherwise the Cottrell current (Equation 2.5) dominates. When performing cyclic voltammetry on UMEs, the scan rate used should be chosen such that a steady-state current can occur, if the scan rate is too high, the behavior is similar to a macro-sized electrode. For a spherical UME, for example, at scan rates

$$v \ll \frac{RTD_O}{nFr_0^2}, \quad (2.7)$$

the steady-state current dominates. For a spherical UME of radius $5 \text{ } \mu\text{m}$ this critical scan rate equals 1 V s^{-1} . Furthermore, at scan rates below this critical scan rate, the peak

current is independent of scan rate. Interestingly, the steady-state current on a UME is proportional to the radius, instead of the area, which is the case at macro-sized electrodes. However, the dependence of the steady state current on the diffusion makes UMEs less suitable for measurements in high-viscosity solvents, such as ionic liquids.^[28] UMEs are conventionally employed in one of the two regions, either linear or steady-state. The properties and characteristics of both macro-sized electrodes and UMEs has led the fabrication of electrodes in microfluidic devices.

2.3 Electrochemistry in microfluidic devices

Miniaturization is an important topic in the development of microfluidic devices, and many candidates for 'Lab on a chip'-applications are using electrochemical techniques.^[29-30] Research was done on fabrication of these devices,^[31-44] integration of electrodes,^[45-55] and the incorporation^[56] and detection^[57-61] of biomolecules.

2.3.1 Fabrication of in-channel electrodes

Lee et al.^[55] integrated both a polymerase chain reaction (PCR) and electrochemical detection for DNA analysis in a single device. The device is shown in Figure 2.5. Platinum heaters and sensors were integrated, as well as platinum (quasi)-reference and counter electrodes. As working electrode, two devices were fabricated, one with gold and one with indium tin oxide (ITO) as working electrode. The reaction chamber of 340 μm depth was etched in silicon, which was bound to the glass electrode wafer using an anodic bonding technique. Using this device, the authors could successfully multiply and subsequently sense the DNA template up to 0.4 fg μl^{-1} .

Figure 2.5: Device photograph (left) and schematics (right) of the PCR-electrochemical device fabricated by Lee et al.^[55] both top (a) and bottom (b) are shown. H1, H2 and S1 to S4 are the platinum heaters (H) and sensors (S), RE and CE the platinum reference and counter electrode, respectively. The working electrode (WE) is gold or ITO. Reproduced from Ref. ^[55] with permission of The Royal Society of Chemistry.

Lee et al.^[47] fabricated semicircular detection electrodes to measure conductivity over a channel. Photographs and a SEM image of their device is shown in Figure 2.6. The fabrication of the semicircular electrode required precise alignment of the cover and channel wafer, as both were patterned with the gold electrode. Using this capacitance-

based detection system, Rhodamine B could be successfully detected via the change in capacitance over the electrodes. The use of the semicircular electrode over the planar electrode increased the sensitivity 4 times.

Figure 2.6: Capacitance-based detection device of Lee et al.^[47] Left image: SEM image of the device, channels are indicated. Right image: Photographs of the device: a) Top view, b) and c) cross-section views of the semicircular electrode and planar electrode, respectively. Reproduced from ^[47] with permission from John Wiley and Sons.

Schrott et al.^[50] have directly integrated electrodes into poly(methyl methacrylate) (PMMA) wafers. By using galvanically deposited gold, the authors circumvented the use of vacuum-driven evaporation techniques.^[51] By employing this technique, a gold layer of 3 μm thickness could be obtained in only 45 minutes. A photograph and the fabrication steps of the device are shown in Figure 2.7.

Figure 2.7: Left image: Photograph of the interdigitated microelectrode array. Inset: close-up, the electrodes are $15\ \mu\text{m}$ in width and $85\ \mu\text{m}$ apart. Right image: Fabrication steps of the device.^[50] Reprinted from ^[50], Copyright 2009, with permission from Elsevier.

This fabrication technique is used in three different testing experiments: An electrolyte diode system using KCl and KOH, a cation exchange membrane and an electroosmotic pump.

In-plane electrodes, in contrast to electrodes in parallel, do not provide linear electric-field lines. To fabricate a device with a homogeneous electric field, Segerink et al.^[48] used a parallel electrode structure. Schematics of the device are shown in Figure 2.8. A homogeneous field is required to increase the sensitivity of the device when sensing individual particles, as inhomogeneities in the electric field results in an unwanted (in-channel) position-dependent impedance when measuring the analyte. Interestingly, both electrodes are deposited in a different way. The floating electrode is fabricated though a shadow mask by direct sputtering onto the glass. The embedded connecting electrode on the top wafer is fabricated through a conventional lift-off technique. To prevent flow from one detection channel to another, UV-curable glue is added to block the channel that is made due to the embedding of the floating electrode.

Figure 2.8: Floating electrode design of Segerink et al.^[48] (a) Side-view of the device, showing the UV-glue blocking cross-talk between the main and side channels. (b) Simplified electronic scheme of the device. (c) Perspective view of the device. Reproduced from Ref. ^[48] with permission of The Royal Society of Chemistry.

Segerink et al.^[48] tested the device using the impedance change induced by polystyrene beads flowing through the channels near the electrodes. The passing of a bead resulted in a peak in impedance of $9.3 \pm 3.6 \, \Omega$ and $12.8 \pm 5.9 \, \Omega$ for 3 and 6 μm beads, respectively.

In a similar study, Janouš et al.^[49] fabricated a PMMA-based sensor with interdigitated electrodes. Photographs and schematics of the device are shown in Figure 2.9. The electrodes were fabricated using galvanic deposition of gold on a phosphor-bronze substrate, as done by Schrott et al.^[50] Prior bonding via a thermal press (80°C, 250 kg), the PMMA is treated with UV-Ozone and isopropanol.

Figure 2.9: PMMA-based microfluidic chip with interdigitated electrodes, from Janouš et al.^[49] (i) Top PMMA plate with interdigitated electrodes. (ii) Electrical connection block through the bottom wafer. (iii) Microfluidic channels in the PMMA. (iv) Electrical connection pads. Reprinted from ^[49], Copyright 2012, with permission from Elsevier.

The characteristics of the device were tested via conductivity studies on various concentrations of NaCl, at various frequencies and showed a linear response of conductance vs. concentration. However, at low frequencies (<200 MHz) the linearity was lost, according to the authors due to the Helmholtz double layer, since the effect of the double layer on the impedance did not vanish completely at lower frequencies.

Laurette et al.^[54] incorporated a gold-coated microchannel in a glass/silicon/glass microfluidic device for miniaturization of terahertz spectroscopy. Photographs of the device are shown in Figure 2.10. To fabricate this device, gold is first evaporated on a glass wafer, and via a lift-off technique the electrodes are formed. Due to the nature of the THz experiment, the authors decided to add silicon sidewalls to the device. Hence, a silicon wafer was first bonded to the glass-electrode system using a polymer-thermocompression process. The silicon wafer is milled down to the appropriate pre-etch size of 180 μm using SF_6 gas. After etching, the glass/silicon stack is bonded to another glass wafer, again using the thermocompression technique.

Figure 2.10: Microfluidic device with integrated electromagnetic and microfluidic functions, by Laurette et al.^[54] Left image: Overview photograph of the device. Right image: Close-up of the gold Goubau line covering the microchannel. © IOP Publishing. Reproduced with permission. All rights reserved.

The finalized electromagnetic / microfluidic device is tested by measuring various concentrations of lysozyme by changing the frequency through the Goubau line, which acts as a waveguide for electromagnetic waves, and guides the waves across the microchannel. The authors found significant increase of the transmission parameter when adding 100 and 180 mg mL^{-1} lysozyme to the water in the microfluidic device, indicating their device worked as intended.

From the examples shown above, it can be seen that two different types of electrodes emerge: one with single in-channel electrodes and one with multiple, interdigitated electrodes. Hence, the design of the microfluidic chip used in this thesis incorporates both types. In this thesis, the two different chips were fabricated on the same glass wafer. The design is shown in Figure 2.11.

Figure 2.11: Design of the electrochemical chip used in this thesis. (a) Design which incorporates one electrode per channel. (b) Design with the interdigitated electrode. Black: channel definition, 50 μm wide and 10 μm wide where the electrodes are. Gold: electrodes, width 100 μm . The interdigitated electrode has a spacing of 500 μm . The circles will be the location of the powderblasted holes. Total size: 22.5 mm by 25 mm.

The chip was finalized by thermal bonding with a cover wafer, on which holes were powderblasted. The step-by-step process can be found in the appendix.

Microfluidic devices with integrated electrodes can be utilized in a plethora of applications. Such as, capillary electrophoreses,^[62-63] potentiometric titrations,^[64] measuring the velocity of on-chip droplets,^[65] digital microfluidics,^[66-67] modeling biological barriers,^[68] particle^[69-70] and liquid handling^[71] and on-chip pumping.^[72] Of particular interest is the detection of (bio)molecules.^[73] In the next paragraph a few examples from the literature are discussed in more detail.

2.3.2 In-chip electrochemical sensing of (bio)molecules

Wang et al.^[56] fabricated a device that could simultaneously sense both glucose and insulin via electrochemical means, as the ratio between these molecules is of important to diabetes patients. The authors found that despite the large difference in concentration, i.e., millimolar range for glucose and nanomolar for insulin, the device responded independently to both molecules. However, to reach these conclusions, their device operates at high voltages, above 1 kV, to reach proper in-chip separation for the electropherograms. Dungchai et al.^[74] detected glucose differently: on a paper-based microfluidic device, the authors attached three different oxidase enzymes, namely glucose oxidase, lactate oxidase and uricase, to three different sets of electrochemistry electrodes. The results of the chronoamperometric experiments for glucose and lactate oxidase are shown in Figure 2.12.

Figure 2.12: Chronoamperograms of two of the three systems explored by Dungchai et al.^[74] a) Electrode with glucose oxidase, b) electrode with lactase oxidase. $E = 0$ V vs. Ag/AgCl. Reprinted with permission from ^[74]. Copyright 2009 American Chemical Society.

From these chronoamperograms could be determined that the limit of detection for this device is 0.21, 0.36 and 1.38 mM for glucose, lactate and uric acid, respectively.

Nie et al.^[75] also used a paper-based device for detection of glucose. Furthermore, they also tested their system by square-wave anodic stripping analysis of heavy metals, such as zinc and lead. The authors found that their device had a higher sensitivity (approximately $1 \mu\text{g kg}^{-1}$) and lower limit of detection compared to conventionally used, macro-sized systems ($2.5 \mu\text{g kg}^{-1}$).

Employing a different technique for detection, García and Henry^[59] detected glucose,^[59] carbohydrates, amino acids and antibiotics^[61] as well as creatinine, creatine and uric acid.^[60] The authors used pulsed amperometry in combination with a two-electrode setup to sense the on-chip separated analytes. Detection limits in the range of femtomolars could be achieved for various biomolecules. Their electrode system, however, were wires inserted into the poly(dimethylsiloxane) (PDMS) device.

Rossier and Girault^[76] fabricated a device with in-channel carbon electrodes as an on-chip enzyme-linked immunosorbent assay (ELISA). Two electrode widths were tested: 15 and 180 μm wide. The authors conclude that the smaller electrode is preferable, due to the steady-state current of an ultramicroelectrode. The device consisted of a photoablated poly(ethylene terephthalate) layer laminated to a poly(ethylene) film.^[77] They employed an antibody linked to horseradish peroxidase to detect D-Dimer, which is the final degradation product produced by blood clotting, in a sandwich immunassay. Using a physisorbed conjugate, the D-Dimer could be detected up to 100 pM, within 15 minutes. Similar work was done by Choi et al.^[78], who placed the antibodies on magnetic beads, so that the microfluidic devices can be flushed and reused at will. They characterized their device using an antibody pair labelled with alkaline phosphatase.

After injection of p-aminophenyl phosphate (PAPP) and subsequent measurement, the chamber was flushed to test the rejuvenating properties of the device. The signal decreased significantly, yet some current remained. The authors contribute this to non-specific binding, magnetic particle clogging or PAPP decomposition.

In order to fabricate a fully integrated microfluidic electrochemical DNA sensor, Ferguson et al.^[79] incorporated symmetric PCR, single-stranded DNA generation and electrochemical detection into a single glass device. As counter and reference electrodes 250 nm platinum was evaporated onto the glass, as working electrode 250 nm gold was used. Both materials had a 20 nm titanium adhesive layer. Methylene blue was labelled to a thiol-ended complementary DNA strand, and used as the probe molecule. AC voltammetry^[80] was used to detect the *Salmonella GyrB* target DNA-strand. The electrochemical results of two concentrations of sample, as well as a blank measurements, are shown in Figure 2.13.

Figure 2.13: a) Blank measurement without sample. b) 100 aM sample yielded 52% decrease in current. c) 10 aM sample resulted in 12% decrease in current. Blue line: baseline measurement, red line: sample measurement, green line: regeneration of probe molecule, purple line: negative control measurement. Reprinted with permission from ^[79]. Copyright 2009 American Chemical Society

A detection limit up to 10 aM of target DNA could be achieved. Post-measurement regeneration of the DNA probe could be realized by rinsing with 8 M guanidine hydrochloride, followed by deionized water. Their device, however, could only be used with a certain DNA strand, meaning new devices have to be fabricated to test other targets. Nevertheless, the sensitivity and ease of use mean this is a good candidate for a point-of-care device.

The detection and sensing of (bio)molecules can be greatly improved using an intermediate layer, such as a polymer. Furthermore, these layers could be used as on-chip valve or pump. Polymers as functional elements in microfluidic devices, as hydrogels, thin layers or polymer brushes, will be discussed in the following chapter. In the following sections, a short introduction on polymer brushes will be given, followed by a review of molecular dynamics simulations on polymer brushes.

2.4 Polymer brushes

When polymer chains are tethered on one end to a substrate at sufficient density, they are called a polymer brushes. The confinement of one end of the polymers leads to new and interesting properties. When the polymers, now attached to a surface, are placed in a poor solvent, the brush collapses. However, when placed in a good solvent, the brush swells due to the favorable solvent-polymer brush interaction. This way the height of the brush can be controlled, as well as a variety of other properties, such as the elastic modulus.^[81] The number of polymers grafted to or from the surface per area is called the grafting density, σ_g . Depending on this grafting density and the solvent, the polymer brush can be in various states, from a regime where the polymers lay flat on the surface (mushroom regime), where every chain does not interact with its neighbor, to a fully stretched brush (high density regime), as shown schematically in Figure 2.14.

Figure 2.14: Schematic representation of the polymer brush regimes, depending on grafting density, σ_g . In the mushroom regime, the distance between grafting spots is above twice the radius of gyration, and the polymer chains do not interact with each other. When the distance is decreased, more brush-like behavior occurs, until the brush is fully stretched in the high-density regime.^[82] Reprinted with permission from ^[82]. Copyright 2011 American Chemical Society.

Since the polymer is a system in thermal equilibrium, it is interesting to know the density at a certain distance from the grafting surface, at various conditions and the height of the brush under these conditions. To this end various theories have been developed. Pioneering in the field were P.G. de Gennes^[83-85] and S. Alexander,^[86] who laid the groundwork for the theoretical study of polymer brushes. In a good solvent, if the distance between grafting spots (D) is above the radius of gyration (R_g),^[87] the density profile is:

$$\phi(z) = \sigma_g \left(\frac{z}{a} \right)^{2/3} \text{ for } a \ll z \ll R_g. \quad (2.8)$$

At distances above the radius of gyration, the drop-off in density is steep. This situation is not very brush-like, if we now move the grafting points closer to each other, such that $D < R_g$, the situation becomes:

$$\phi \sim \sigma_g^{2/3} \quad (2.9)$$

in the brush far from the wall. At the wall, there is a depletion layer similar to Equation (2.8). From this the thickness H of the brush can be derived:

$$H \sim Na\sigma_g^{1/3}. \quad (2.10)$$

Interestingly, De Gennes suggests a sharp step-like drop-off of the brush at H . Milner et al.^[88] suggest otherwise. Using self-consistent field (SCF) theory, they calculated that a polymer brush has a parabolic density profile near the end of the brush. The scaling theory of Alexander-De Gennes (Equation 2.10) was refined to:

$$H = N \left(\frac{12\sigma_g a}{\pi^2} \right)^{1/3}, \quad (2.11)$$

this corresponds to the above mentioned parabolic density profile:

$$\phi(z) = \frac{\pi^2}{8aN^2} (H^2 - z^2). \quad (2.12)$$

This parabolic density profile can be verified by the precise study of the location of the monomers within a polymer chain, for which molecular dynamics simulations can be employed.

2.5 Molecular dynamics simulations

Molecular dynamics (MD) simulation is a simulation of a many-body system, during which one can monitor the equilibrium and transport properties of the system. Polymer brushes can be studied on the monomer,^[89] or even atomic level.^[90] In its simplest sense, a MD simulation solves Newton's equations of motion, and does so over time. As the number of particles increases, so does the computational time. From the location and momentum of the particles, observables, such as density and temperature, can be calculated.

2.5.1 Basics of molecular dynamics simulations

In MD simulations, the dispersive interaction between the particles is often governed by the Lennard-Jones (LJ) potential:

$$V_{LJ} = 4\epsilon \left(\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right), \quad (2.13)$$

which has a potential depth of ϵ , and σ is the distance between two particles (r_{ij}) at which $V_{LJ} = 0$. The equilibrium distance, at which the forces between the particles equals zero, is at $r_{ij} = 2^{1/6} \sigma$. By evaluating the LJ potential between all particles, the forces on the individual particles are known. MD results are often displayed in reduced LJ units, which are derived directly from this potential: σ for distance, ϵ for energy. These units can be related to real values, for example, for poly(ethylene): $\sigma = 0.5$ nm and $\epsilon = 30$ meV.^[91]

To save computational time, in most, if not all, MD simulations a cut-off is used for the LJ potential, usually 2.5σ . To further reduce the CPU time required, neighbor lists can be employed, where the interaction potential is only evaluated for a particle n with a list of particles m . This list is made at the start of the simulation, and contains those particles that have $r_{nm} < 2.5 \sigma$. Naturally, this list needs to be updated during the simulation, resulting in an increase of CPU time. Nevertheless, if the system contains more than 100 particles, using a neighbor list is beneficial.^[92]

To calculate the particle position at the next simulation step, one integrates the acceleration of the particle over time, to get the velocity of the particle. Via the velocity the position of the particle at the next step can be determined. However, in MD simulations, everything is discretized, hence various algorithms have been developed to minimize the error in the velocity calculation. The simplest method is called the Euler method:

$$r(t + \Delta t) = r(t) + v(t)\Delta t, \quad (2.14)$$

which is straightforward, yet not energetically stable in many MD simulations. Another algorithm is the Leap Frog algorithm, which evaluates the velocities at half time steps:

$$v(t \pm \Delta t/2) = \frac{\mp r(t) \pm r(t \pm \Delta t)}{\Delta t}. \quad (2.15)$$

This algorithm is more energetically stable, however, since the velocities are calculated at a different time from the position, the kinetic and potential energy are not known at these time intervals. An algorithm that circumvent this issue is the Verlet algorithm,^[93] which takes the Taylor expansion around the coordinate of the particle:

$$r(t \pm \Delta t) = r(t) \pm v(t)\Delta t + \frac{f(t)}{2m}\Delta t^2 \pm \frac{\Delta t^3}{3!}\ddot{r}(t) + \dots, \quad (2.16)$$

$$r(t + \Delta t) + r(t - \Delta t) = 2r(t) + \frac{f(t)}{m}\Delta t^2 + \dots, \quad (2.17)$$

$$r(t + \Delta t) \approx 2r(t) - r(t - \Delta t) + \frac{f(t)}{m}a(t)\Delta t^2. \quad (2.18)$$

The Verlet algorithm calculates the new position without the velocity. This decoupling of position and velocity leads to an energetically stable simulation.^[92] Naturally the velocity can be calculated using:

$$v(t) = \frac{r(t + \Delta t) - r(t - \Delta t)}{2\Delta t}. \quad (2.19)$$

This gives a velocity accuracy of order Δt^2 . The Verlet algorithm calculates the position and velocity at different time steps. It is possible to rearrange the Verlet algorithm into a form that does calculate the position and velocity at the same time:

$$r(t + \Delta t) = r(t) + v(t)\Delta t + \frac{f(t)}{2m}\Delta t^2, \quad (2.20)$$

$$v(t + \Delta t) = v(t) + \frac{f(t + \Delta t) + f(t)}{2m}\Delta t, \quad (2.21)$$

and this is called the velocity-Verlet scheme. More accurate algorithms are known, such as a Runge-Kutta method, but these take more computational time for only a little increase in accuracy, hence most MD simulations use the velocity-Verlet algorithm.

Through the kinetic energy of the particles, the temperature in a MD simulation can be calculated, since:

$$3(N - 1)k_B T = \sum_{i=1}^N m_i v_i^2. \quad (2.22)$$

Where $3(N - 1)$ is the number of degrees of freedom with 3 constraints. To control the temperature, one can modify the velocity. Often, for the initial state of the simulation, the temperature is manually set to a certain value. However, manually modifying particle properties in a running simulation should be done with great care not to disturb the measurement. For the purpose of temperature-control, several methods have been developed.

By using the Berendsen thermostat,^[94] the system is in equilibrium with an external mathematical heat bath. The temperature control is such that when a deviation of the set temperature occurs, the system is forced by exponential decay back to the set temperature. Another way to simulate a heat bath is done by the Andersen thermostat.^[95] The Andersen thermostat creates a momentum distribution around the set temperature, and randomly replaces a particles' momentum with one from the distribution. This approach is more on a per particle basis. However, this approach results in memory loss of molecular kinetics. A third way to incorporate a heat bath into a MD simulation is the Nose-Hoover thermostat.^[96-97] This thermostat adds a thermal term to the system's Hamiltonian, resulting in control over the temperature through the particles' momenta.

By using a Langevin thermostat,^[98] individual particles are slowed down according to the set temperature as if they were immersed in a solvent, incorporation both viscous drag and collisions with the solvent molecules. This thermostat is most useful for systems with long equilibration times. Dissipative particle dynamics (DPD),^[99] is similar to a Langevin thermostat, only that instead of damping the velocity of a particle, the damping is enforced on pair-wise interactions with other particles, i.e. the thermostat is active only if a particle interacts with another. Due to this fact, DPD is most useful in systems in motion. Regardless of the choice of thermostat, each employs a type of damping, and care should be taken that the correct damping factor is set.

2.5.2 Molecular dynamics simulations on polymer brushes

For simulations on polymer brushes, a coarse-grained model first proposed by Kremer and Grest is often used.^[100] This model assumes the individual monomers to be spheres, which are connected via springs to form a polymer chain. These 'springs' are governed by a different potential:

$$V_{\text{FENE}} = -0.5kR_0^2 \ln \left(1 - \left(\frac{r_{ij}}{R_0} \right)^2 \right), \quad (2.23)$$

also known as the finite-extensible nonlinear elastic potential, which is added to the LJ potential. When $k = 30 \epsilon \sigma^{-2}$ and $R_0 = 1.5 \sigma$, no bond crossing occurs.^[100] To simulate a good solvent, instead of adding solvent particles, the LJ potential is shifted upwards by ϵ , and cut off at $r_{ij} = 2^{1/6} \sigma$, resulting in repulsive interactions between monomers, leading to the stretching of the brush as it would in a good solvent.

As shown in paragraph 2.4, from SCF theory can be calculated that a brush assumes a parabolic density profile. By employing MD simulations, the actual density profiles, under various conditions, can be determined. Murat and Grest^[101] resolved the density profile of a polymer brush of various length at surface coverages from 0.01 to 0.2 chains σ^{-2} . They found that both the scaling theory of Alexander-de Gennes^[84,86] and the SCF theory of Milner-Witten-Cates^[88] is only valid up to grafting densities of 0.1 chains σ^{-2} for brushes of length $N = 50$ and 0.03 chains σ^{-2} for length $N = 100$ when comparing the first moment of the polymer brush density (which is defined as the average brush thickness). The authors attributed the discrepancies of the simulations at higher chain length and σ_g with the theory to the way the higher densities are obtained, as Milner et al.^[88] increase the density by varying the monomer interaction parameters, in contrast to the authors, who actually reduce the number of chains per surface area.

The simulations of Murat and Grest^[101] assume a good solvent for the polymer brush. Binder et al.^[102] varied the polymer-solvent interaction and studied, among other things, the change in density of both the solvent and the polymer brush. Figure 2.15 shows the results of their simulation. ϵ_{PP} and ϵ_{PS} are the energies with which the Lennard-Jones interaction potential is shifted down, for polymer-polymer interactions and polymer-solvent interactions, respectively. If $\epsilon_{\text{PP}} = 0$ and $\epsilon_{\text{PS}} = 0$, the solvent is a good solvent for the polymer. In this regime, the parabolic profile of Milner et al.^[88] is found, apart from the rounded low-density region at the top of the brush. In the regime where the polymer-polymer interactions are favored over the solvent-polymer interactions ($\epsilon_{\text{PP}} = 0.8$, $\epsilon_{\text{PS}} = 0$), a more step-wise profile can be observed.

Figure 2.15: Monomer density (top) and solvent density (bottom) at various shifts in interaction potential energies, when the ϵ is increased, the interaction becomes more favorable. $\epsilon_{PP} = 0$ and $\epsilon_{PS} = 0$ simulates a good solvent. Favorable polymer-polymer interaction gives rise to a step function of the density, a good solvent gives a parabolic density profile.^[102] Reproduced from Ref. ^[102] with permission of The Royal Society of Chemistry.

Now that the polymer-brush density-profile can be resolved, more complex studies have been performed. Such as the transition from the mushroom regime towards the stretched brush regime. Egorov et al.^[103] used SCF theory, Monte Carlo simulations and MD simulations to resolve the brush-mushroom cross-over in a good solvent. The authors found that the persistence length, l_p , of the polymer has a significant effect on the exact position of the brush-mushroom cross-over. For fully flexible chains, the cross-over grafting density, σ_{mb} , is $\pi^{-1} R_g^{-2}$. If a chain is semi-flexible, the chains are allowed to overlap more in the mushroom regime before the chains start to stretch normal to the surface, resulting in a positive shift in cross-over grafting density up to a grafting density $\sigma'_{mb} \sim l_p N^{-1} a^{-2.5}$.

Of particular interest in polymer-brush studies is the insertion of nano-sized particles in the brush. Merlitz et al.^[104] used MD to simulate insertion of a spherical particle of size 1 to 5 σ into a polymer brush, with a degree of polymerization of $N = 64$, grafted at a density of 0.1 to 0.4 chains σ^{-2} . The authors measured the force the polymer brush exerted on the particle as function of distance from the substrate, shown in Figure 2.16, when varying the grafting density and when varying the particle size.

Figure 2.16: Vertical force exerted by a polymer brush on (a) a nanoparticle of size $d = 3$ at various grafting densities and on (b) nanoparticles of various size at a grafting density of 0.3 chains σ^2 . It can be seen that an increase of either size or grafting density increases the force accordingly. Furthermore, an increase of grafting density also shifts the peak location farther from the grafting substrate.^[104] Reprinted with permission from ^[104]. Copyright 2012 American Chemical Society.

From the results it can be seen that both increase in the grafting density and increase in the particle size show an increase in the vertical force. However, only the change in grafting density effects the location of the force-maximum, apart from what can be expected by increasing the size of the particle, since larger particles are in contact with the brush at higher distance from the substrate than smaller particles. From their work could be concluded that the peak force scales linearly with the osmotic pressure of the brush, when the particle size is kept constant. When varying the nanoparticle size, a power law scaling on the inclusion free energy arises, however, a conclusive quantitative agreement with models could not be reached.

Not restricting oneself to a single particle, Yaneva et al.^[105] simulated 100 particles, and measured the density profiles of both the brush and the particles as function of nanoparticle size. The authors were interested in the penetration depth of the nanoparticles. An explicit solvent was simulated, in contrast to the results discussed earlier in this chapter, since explicit solvent requires a lot more computational time due to the large increase in particles in the system. The radii of the nanoparticles were varied from 0.5 to 1.7σ . The resulting density profiles are shown in Figure 2.17. From this figure can be deduced that particles of smaller size penetrate deeper into the brush, as can be seen from the nanoparticle density profile, as well as the overlap of the density profiles of the brush and the nanoparticles. When varying both the grafting density and the brush length, a power-law relation of slope -3 could be found between the overlap of the density profiles and the size of the nanoparticle, normalized by a critical particle size b^* , which is the radius below which the particles distribute freely in the brush. This power law was unaffected by brush length.

Figure 2.17: (a) Density profiles of both brush (ϕ_p) and particles (ϕ_{nano}) as function of distance from the grafting wall z (in σ). A schematic of the brush-particle system is shown in the top of the figure. (b) Overlap density profile ($\phi_p\phi_{\text{nano}}$) as function of the distance from the grafting wall z . Brush characteristics: $N = 60$ and $\sigma_g = 0.185$ chains σ^{-2} .^[105] Reprinted from ^[105], Copyright 2009, with permission from Elsevier.

To investigate the effect of size and shape on flow-characteristics of molecules in a brush-covered microchannel, Neratova et al.^[106], set up the following MD simulations: Star polymers with p polymer arms were used as the macromolecules studied, the total number of monomers was kept constant at $N = 151$, resulting in arms lengths of 75, 30, 15, 10 and 5 for the number of arms p of 2, 5, 10, 15, 30, respectively. Note that for $p = 2$, the polymer is linear with a total length of 151 monomers. These star polymers were pushed through a brush-covered microchannel ($N = 30$, $\sigma_g = 2.3 \pi^{-1} R_g^{-2}$), together with solvent particles, by a body force B on all particles except brush ends. The B used was 0.01, 0.1, 0.15, 0.2, 0.3 and $0.36 \epsilon \sigma^{-1}$. The density profiles found are shown in Figure 2.18.

Figure 2.18: Current density profiles of the inclusions, as function of distance within the channel cross-section. The number of arms range from 2 to 30. (a) $B = 0.1 \epsilon \sigma^{-1}$, (b) $B = 0.15 \epsilon \sigma^{-1}$, (c) $B = 0.3 \epsilon \sigma^{-1}$. Note that the y-axis are different from one another.^[106] Reprinted with permission from ^[106]. Copyright 2015 American Chemical Society.

From the density profiles can be concluded that the conformation of the polymer particle has little influence on the density distribution, for the conformations studied. The influence of B is much more pronounced. Furthermore, the mass transfer through the channel was also more influenced by B than the shape of the polymer particle. The authors contribute

this mainly to the conformational changes of the inclusions, since linear chains have more ways to fold in the direction of the flow than rigid star-molecules.

To conclude, MD simulations of polymeric systems give great insight into the effect of grafting density and chain length on the brush dynamics, including height and stiffness.^[107] Furthermore, MD is a powerful tool to study the effect of particles inserted into polymer systems because various properties of the system can be directly extracted from the simulation, such as the density profiles and the exact location of the individual monomers, solvent molecules or particles as function of time. Moreover, MD simulations are a controlled environment where parameters, such as chain length or grafting density, can be structurally varied, leading to insight in polymer properties at the monomer or even atomic level.

Table 2.1: List of symbols used.

Symbol	Description
i	Current (A)
E	Potential (V)
E^0	Formal redox potential (V)
i_p	Peak current (A)
n	Number of electrons in redox reaction
A	Area of the electrode (m^2)
C	Concentration (mol m^{-3})
D	Diffusion coefficient ($\text{m}^2 \text{s}^{-1}$)
v	Scan rate (V s^{-1})
F	Faraday's constant (96485 C mol^{-1})
R	Gas constant ($8.3145 \text{ J K}^{-1} \text{ mol}^{-1}$)
T	Temperature (K)
Γ_0^*	Surface coverage of redox species (m^{-2})
b_X	Langmuir adsorption isotherm of redox species X
Q	Charge (C)
t	Time (s)
m_0	Geometry-dependent mass-transfer coefficient of the steady-state current
r_0	Smallest dimension of an UME (m)
ϕ	Monomer density (m^{-3})
z	Distance to the wall (m)
σ	Grafting density (chains m^{-2})
a	Monomer size (m)
R_g	Radius of gyration (m)
D	Distance between grafting spots (m)
H	Brush height (m)
N	Number of monomers
V_{LJ}	Lennard-Jones interaction potential
ε	Depth of Lennard-Jones potential (J)
σ	Distance at which the Lennard-Jones potential is zero (m)
r_{ij}	Inter-particle distance (σ)
v	Particle velocity ($\sigma \tau^{-1}$)
f	Force on particle ($\varepsilon \sigma^{-1}$)
m	Mass of particle (kg)
k_B	Boltzmann's constant ($1.38 \cdot 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$)
V_{FENE}	Finite extensible nonlinear elastic potential for consecutive monomers
k	Stiffness of the potential ($\varepsilon \sigma^{-2}$)
R_0	Maximum extend of the bond (σ)
l_p	Persistence length of a polymer chain (m)

2.6 References

- [1] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*. John Wiley & Sons, Inc.: **2001**; p 864.
- [2] J. J. Gooding, *Electroanalysis* **2002**, *14* (17), 1149-1156 | 10.1002/1521-4109(200209)14:17<1149::aid-elan1149>3.0.co;2-8
- [3] J. Wang, *Anal. Chim. Acta* **2002**, *469* (1), 63-71 | 10.1016/s0003-2670(01)01399-x
- [4] A. M. Pisoschi, A. Pop, A. I. Serban, C. Fafaneata, *Electrochim. Acta* **2014**, *121*, 443-460 | 10.1016/j.electacta.2013.12.127
- [5] J. Wang, *Chem. Rev.* **2008**, *108* (2), 814-25 | 10.1021/cr068123a
- [6] N. M. Markovic, *Nat Mater* **2013**, *12* (2), 101-2 | 10.1038/nmat3554
- [7] M. Sadakane, E. Steckhan, *Chem. Rev.* **1998**, *98* (1), 219-238 | 10.1021/cr960403a
- [8] S. Wang, F. Xie, G. Liu, *Talanta* **2009**, *77* (4), 1343-50 | 10.1016/j.talanta.2008.09.019
- [9] C. Bunte, O. Prucker, T. Konig, J. Ruhe, *Langmuir* **2010**, *26* (8), 6019-27 | 10.1021/la9037183
- [10] N. C. Foulds, C. R. Lowe, *Anal. Chem.* **1988**, *60* (22), 2473-2478 | 10.1021/ac00173a008
- [11] Y. Xiao, F. Patolsky, E. Katz, J. F. Hainfeld, I. Willner, *Science* **2003**, *299* (5614), 1877-81 | 10.1126/science.1080664
- [12] M. G. Friedrich, V. U. Kirste, J. Zhu, R. B. Gennis, W. Knoll, R. L. Naumann, *J. Phys. Chem. B* **2008**, *112* (10), 3193-201 | 10.1021/jp709717k
- [13] D. J. Kosman, *Mol. Microbiol.* **2003**, *47* (5), 1185-1197 | 10.1046/j.1365-2958.2003.03368.x
- [14] V. S. Bagotsky, *J. Solid State Electrochem.* **2011**, *15* (7-8), 1559-1562 | 10.1007/s10008-010-1281-8
- [15] B. C. Steele, A. Heinzl, *Nature* **2001**, *414* (6861), 345-52 | 10.1038/35104620
- [16] M. Winter, R. J. Brodd, *Chem. Rev.* **2004**, *104* (10), 4245-4270 | 10.1021/cr020730k
- [17] R. He, M. Echeverri, D. Ward, Y. Zhu, T. Kyu, *J. Membr. Sci.* **2016**, *498*, 208-217 | 10.1016/j.memsci.2015.10.008
- [18] K. D. Kreuer, *Solid State Ionics* **1997**, *97* (1-4), 1-15 | 10.1016/s0167-2738(97)00082-9
- [19] T. E. Springer, *J. Electrochem. Soc.* **1991**, *138* (8), 2334 | 10.1149/1.2085971
- [20] N. Wagner, T. Kaz, K. A. Friedrich, *Electrochim. Acta* **2008**, *53* (25), 7475-7482 | 10.1016/j.electacta.2008.01.084
- [21] K. B. Oldham, J. C. Myland, *Electrochim. Acta* **2011**, *56* (28), 10612-10625 | 10.1016/j.electacta.2011.05.044
- [22] J. E. B. Randles, *Transactions of the Faraday Society* **1948**, *44*, 327 | 10.1039/tf9484400327
- [23] A. Ševčík, *Collect. Czech. Chem. Commun.* **1948**, *13*, 349-377 | 10.1135/cccc19480349
- [24] C. E. D. Chidsey, C. R. Bertozzi, T. M. Putvinski, A. M. Muijsce, *J. Am. Chem. Soc.* **1990**, *112* (11), 4301-4306 | 10.1021/ja00167a028
- [25] F. G. Cottrell, *Z. Physik, Chem.* **1902**, *42*, 385 |
- [26] M. Péter. Engineering of surfaces with organometallic poly(ferrocenylsilanes). PhD thesis, University of Twente, Enschede, The Netherlands, **2002**.

- [27] A. E. Kaifer, M. Gómez-Kaifer, *Supramolecular Electrochemistry*. Wiley-VCH Verlag GmbH: **1999**.
- [28] C. L. Bentley, A. M. Bond, A. F. Hollenkamp, P. J. Mahon, J. Zhang, *Anal. Chem.* **2013**, *85* (4), 2239-45 | 10.1021/ac303042r
- [29] W. R. Vandaveer, S. A. Pasas-Farmer, D. J. Fischer, C. N. Frankenfeld, S. M. Lunte, *Electrophoresis* **2004**, *25*, 3528-49 | 10.1002/elps.200406115
- [30] M. A. Schwarz, P. C. Hauser, *Lab on a chip* **2001**, *1*, 1-6 | 10.1039/b103795c
- [31] P. A. J. Kenis, R. F. Ismagilov, G. M. Whitesides, *Science* **1999**, *285* (5424), 83-85 | 10.1126/science.285.5424.83
- [32] J. Wang, M. P. Chatrathi, B. Tian, *Anal. Chem.* **2000**, *72*, 5774-8 | 10.1021/ac0006371
- [33] G. Chen, J. Wang, *The Analyst* **2004**, *129*, 507-11 | 10.1039/b402816c
- [34] D. Daniel, I. G. R. Gutz, *Electrochem. Commun.* **2003**, *5*, 782-786 | 10.1016/j.elecom.2003.07.004
- [35] P. Ertl, C. A. Emrich, P. Singhal, R. A. Mathies, *Anal. Chem.* **2004**, *76* (13), 3749-3755 | 10.1021/ac035282a
- [36] Y. Liu, J. A. Vickers, C. S. Henry, *Anal. Chem.* **2004**, *76*, 1513-7 | 10.1021/ac0350357
- [37] J. Wang, M. Pumera, *Anal. Chem.* **2002**, *74*, 5919-23 | 10.1021/ac020416q
- [38] R. Wilke, S. Büttgenbach, *Biosens. Bioelectron.* **2003**, *19*, 149-153 | 10.1016/S0956-5663(03)00178-7
- [39] C.-C. Wu, R.-G. Wu, J.-G. Huang, Y. C. Lin, C. Hsien-Chang, *Anal. Chem.* **2003**, *75*, 947-52 | 10.1021/ac025912t
- [40] A. Yamaguchi, P. Jin, H. Tsuchiyama, T. Masuda, K. Sun, S. Matsuo, H. Misawa, *Anal. Chim. Acta* **2002**, *468*, 143-152 | 10.1016/S0003-2670(02)00634-7
- [41] J. Yan, Y. Du, J. Liu, W. Cao, X. Sun, W. Zhou, X. Yang, E. Wang, *Anal. Chem.* **2003**, *75*, 5406-5412 | 10.1021/ac034017m
- [42] N. E. Hebert, W. G. Kuhr, S. A. Brazill, *Anal. Chem.* **2003**, *75*, 3301-3307 | 10.1021/ac0262457
- [43] C.-C. Joseph Lai, C.-h. Chen, F.-H. Ko, *J. Chromatogr. A* **2004**, *1023* (1), 143-150 | 10.1016/j.chroma.2003.09.053
- [44] Y. Liu, J. C. Fanguy, J. M. Bledsoe, C. S. Henry, *Anal. Chem.* **2000**, *72*, 5939-44 | 10.1021/ac000932l
- [45] A. Berthold, F. Laugere, H. Schellevis, C. R. de Boer, M. Laros, R. M. Guijt, P. M. Sarro, M. J. Vellekoop, *Electrophoresis* **2002**, *23* (20), 3511-9 | 10.1002/1522-2683(200210)23:20<3511::AID-ELPS3511>3.0.CO;2-C
- [46] E. D. Goluch, B. Wolfrum, P. S. Singh, M. A. G. Zevenbergen, S. G. Lemay, *Anal. Bioanal. Chem.* **2009**, *394*, 447-56 | 10.1007/s00216-008-2575-x
- [47] C.-Y. Lee, C. M. Chen, G.-L. Chang, C.-H. Lin, L.-M. Fu, *Electrophoresis* **2006**, *27*, 5043-5050 | 10.1002/elps.200600113
- [48] L. I. Segerink, A. J. Sprenkels, J. G. Bomer, I. Vermes, A. van den Berg, *Lab on a chip* **2011**, *11*, 1995-2001 | 10.1039/c0lc00489h
- [49] J. Janouš, P. Beránek, M. Přibyl, D. Šnita, *Microelectron. Eng.* **2012**, *97*, 387-390 | 10.1016/j.mee.2012.05.008
- [50] W. Schrott, M. Svoboda, Z. Slouka, D. Šnita, *Microelectron. Eng.* **2009**, *86*, 1340-1342 | 10.1016/j.mee.2009.01.001
- [51] M. Svoboda, Z. Slouka, W. Schrott, P. Červenka, M. Přibyl, D. Šnita, *Microelectron. Eng.* **2010**, *87*, 1590-1593 | 10.1016/j.mee.2009.11.010

- [52] C. J. Easley, L. A. Legendre, M. G. Roper, T. A. Wavering, J. P. Ferrance, J. P. Landers, *Anal. Chem.* **2005**, 77, 1038-1045 | 10.1021/ac048693f
- [53] A. Hilmi, J. H. T. Luong, *Anal. Chem.* **2000**, 72, 4677-4682 | 10.1021/ac000524h
- [54] S. Laurette, A. Treizebre, B. Bocquet, *J. Micromech. Microeng.* **2011**, 21, 065029 | 10.1088/0960-1317/21/6/065029
- [55] T. M.-H. Lee, M. C. Carles, I.-M. Hsing, *Lab on a chip* **2003**, 3, 100 | 10.1039/b300799e
- [56] J. Wang, A. Ibáñez, M. P. Chatrathi, *J. Am. Chem. Soc.* **2003**, 125, 8444-5 | 10.1021/ja036067e
- [57] M. A. Schwarz, P. C. Hauser, *Anal. Chem.* **2003**, 75, 4691-5 | 10.1021/ac030148b
- [58] J. Wang, M. P. Chatrathi, *Anal. Chem.* **2003**, 75, 525-9 | 10.1021/ac020560b
- [59] C. García, C. S. Henry, *Anal. Chim. Acta* **2004**, 508, 1-9 | 10.1016/j.aca.2003.11.060
- [60] C. D. Garcia, C. S. Henry, *The Analyst* **2004**, 129, 579-84 | 10.1039/b403529a
- [61] C. D. García, C. S. Henry, *Anal. Chem.* **2003**, 75 (18), 4778-83 | 10.1021/ac034440v
- [62] Y.-C. Lin, W.-D. Wu, *Sensors Actuators B: Chem.* **2001**, 73 (1), 54-62 | 10.1016/s0925-4005(00)00680-8
- [63] A. A. D. Bani-Yaseen, *IEEE Sens. J.* **2009**, 9 (2), 81-86 | 10.1109/jsen.2008.2011074
- [64] R. Ferrigno, J. N. Lee, X. Jiang, G. M. Whitesides, *Anal. Chem.* **2004**, 76 (8), 2273-80 | 10.1021/ac035281i
- [65] S. Liu, Y. Gu, R. B. Le Roux, S. M. Matthews, D. Bratton, K. Yunus, A. C. Fisher, W. T. Huck, *Lab on a chip* **2008**, 8 (11), 1937-42 | 10.1039/b809744e
- [66] S. K. Cho, H. Moon, C.-J. Kim, *Journal of Microelectromechanical Systems* **2003**, 12 (1), 70-80 | 10.1109/jmems.2002.807467
- [67] L. Chen, R. B. Fair, *Microfluid. Nanofluid.* **2015**, 19 (6), 1349-1361 | 10.1007/s10404-015-1650-9
- [68] F. R. Walter, S. Valkai, A. Kincses, A. Petneházi, T. Czeller, S. Veszeka, P. Ormos, M. A. Deli, A. Dér, *Sensors Actuators B: Chem.* **2016**, 222, 1209-1219 | 10.1016/j.snb.2015.07.110
- [69] S. E. Yalcin, A. Sharma, S. Qian, S. W. Joo, O. Baysal, *Electrophoresis* **2010**, 31 (22), 3711-8 | 10.1002/elps.201000330
- [70] M. Zhang, Y. Ai, A. Sharma, S. W. Joo, D. S. Kim, S. Qian, *Electrophoresis* **2011**, 32 (14), 1864-74 | 10.1002/elps.201100050
- [71] J. Lee, H. Moon, J. Fowler, T. Schoellhammer, C.-J. Kim, *Sensors and Actuators A: Physical* **2002**, 95 (2-3), 259-268 | 10.1016/s0924-4247(01)00734-8
- [72] A. Ajdari, *Physical Review E* **2000**, 61 (1), R45-R48 | 10.1103/PhysRevE.61.R45
- [73] S. Mross, S. Pierrat, T. Zimmermann, M. Kraft, *Biosens. Bioelectron.* **2015**, 70, 376-91 | 10.1016/j.bios.2015.03.049
- [74] W. Dungchai, O. Chailapakul, C. S. Henry, *Anal. Chem.* **2009**, 81 (14), 5821-6 | 10.1021/ac9007573
- [75] Z. Nie, C. A. Nijhuis, J. Gong, X. Chen, A. Kumachev, A. W. Martinez, M. Narovlyansky, G. M. Whitesides, *Lab on a chip* **2010**, 10 (4), 477-83 | 10.1039/b917150a

- [76] J. S. Rossier, H. H. Girault, *Lab on a chip* **2001**, 1 (2), 153-7 | 10.1039/b104772h
- [77] J. S. Rossier, M. A. Roberts, R. Ferrigno, H. H. Girault, *Anal. Chem.* **1999**, 71 (19), 4294-9 | 10.1021/ac981382i
- [78] J.-W. Choi, K. W. Oh, A. Han, C. A. Wijayawardhana, C. Lannes, S. Bhansali, K. T. Schlueter, W. R. Heineman, H. B. Halsall, J. H. Nevin, A. J. Helmicki, H. T. Henderson, C. H. Ahn, *Biomed. Microdevices* **2001**, 3 (3), 191-200 | 10.1023/a:1011490627871
- [79] B. S. Ferguson, S. F. Buchsbaum, J. S. Swensen, K. Hsieh, X. Lou, H. T. Soh, *Anal. Chem.* **2009**, 81 (15), 6503-8 | 10.1021/ac900923e
- [80] A. M. Bond, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1974**, 50 (2), 285-291 | 10.1016/s0022-0728(74)80160-9
- [81] A. Li, E. M. Benetti, D. Tranchida, J. N. Clasohm, H. Schönherr, N. D. Spencer, *Macromolecules* **2011**, 44 (13), 5344-5351 | 10.1021/ma2006443
- [82] L. C. Moh, M. D. Losego, P. V. Braun, *Langmuir* **2011**, 27 (7), 3698-702 | 10.1021/la2002139
- [83] P. G. de Gennes, *Scaling Concepts in Polymer Physics*. Cornell University Press: **1979**.
- [84] P. G. de Gennes, *Macromolecules* **1980**, 13, 1069 | 10.1021/ma60077a009
- [85] P. G. de Gennes, *Adv. Colloid Interface Sci.* **1987**, 27 (3-4), 189-209 | 10.1016/0001-8686(87)85003-0
- [86] S. Alexander, *J. Phys.-Paris* **1977**, 38 (8), 983 | 10.1051/jphys:01977003808098300
- [87] P. J. Flory, *J. Chem. Phys.* **1942**, 10, 51 | 10.1063/1.1723621
- [88] S. T. Milner, T. A. Witten, M. E. Cates, *Macromolecules* **1988**, 21 (8), 2610 | 10.1021/Ma00186a051
- [89] D. Reith, A. Milchev, P. Virnau, K. Binder, *Macromolecules* **2012**, 45 (10), 4381-4393 | 10.1021/ma202745b
- [90] J. D. Perlmutter, W. J. Drasler, 2nd, W. Xie, J. Gao, J. L. Popot, J. N. Sachs, *Langmuir* **2011**, 27 (17), 10523-37 | 10.1021/la202103v
- [91] K. Kremer, G. S. Grest, *The Journal of Chemical Physics* **1990**, 92 (8), 5057 | 10.1063/1.458541
- [92] D. Frenkel, B. Smit, *Understanding Molecular Simulation*. Academic Press: London, **2002**; p 638.
- [93] L. Verlet, *Physical Review* **1967**, 159 (1), 98-103 | 10.1103/PhysRev.159.98
- [94] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. DiNola, J. R. Haak, *The Journal of Chemical Physics* **1984**, 81 (8), 3684 | 10.1063/1.448118
- [95] H. C. Andersen, *The Journal of Chemical Physics* **1980**, 72 (4), 2384 | 10.1063/1.439486
- [96] S. Nosé, *The Journal of Chemical Physics* **1984**, 81 (1), 511 | 10.1063/1.447334
- [97] W. G. Hoover, *Physical Review A* **1985**, 31 (3), 1695-1697 | 10.1103/PhysRevA.31.1695
- [98] T. Schneider, E. Stoll, *Physical Review B* **1978**, 17 (3), 1302-1322 | 10.1103/PhysRevB.17.1302
- [99] P. J. Hoogerbrugge, J. M. V. A. Koelman, *Europhysics Letters (EPL)* **1992**, 19 (3), 155-160 | 10.1209/0295-5075/19/3/001
- [100] G. S. Grest, K. Kremer, *Phys. Rev. A* **1986**, 33 (5), 3628 | 10.1103/PhysRevA.33.3628

- [101] M. Murat, G. S. Grest, *Macromolecules* **1989**, 22 (10), 4054-4059 | 10.1021/ma00200a041
- [102] K. Binder, T. Kreer, A. Milchev, *Soft Matter* **2011**, 7 (16), 7159 | 10.1039/c1sm05212h
- [103] S. A. Egorov, H. P. Hsu, A. Milchev, K. Binder, *Soft Matter* **2015**, 11 (13), 2604-16 | 10.1039/c4sm02862g
- [104] H. Merlitz, C.-X. Wu, J.-U. Sommer, *Macromolecules* **2012**, 45 (20), 8494-8501 | 10.1021/ma301781b
- [105] J. Yaneva, D. I. Dimitrov, A. Milchev, K. Binder, *J. Colloid Interface Sci.* **2009**, 336 (1), 51-8 | 10.1016/j.jcis.2009.03.062
- [106] I. V. Neratova, T. Kreer, J.-U. Sommer, *Macromolecules* **2015**, 48 (11), 3756-3766 | 10.1021/acs.macromol.5b00042
- [107] S. M. Balko, T. Kreer, P. J. Costanzo, T. E. Patten, A. Johnner, T. L. Kuhl, C. M. Marques, *PLoS ONE* **2013**, 8, e58392 | 10.1371/journal.pone.0058392.t001

Stimulus-responsive polymers and other functional polymer surfaces as components in glass microfluidic channels

In this chapter, the application of polymers in glass microfluidic devices is reviewed. The integration of smart, stimulus-responsive polymers as functional elements within microfluidic devices has greatly improved the performance capabilities of controlled fluid delivery. For their use as actuators in microfluidic systems, reversible expansion and shrinking are unique mechanisms which can be utilized as both, passive and active fluid control elements to establish gate and valve functions (passive) and pumping elements (active). Various constituents in microfluidic glass channels based on stimulus-responsive elements have been reported, based on pH-responsive, thermoresponsive and photoresponsive coatings. Fluid control and robust performance have been demonstrated in microfluidic devices in a number of studies. Here we give a brief overview of selected examples from the literature reporting on the use of stimulus-response polymers as active or passive elements for fluid control in microfluidic devices, with specific emphasis on glass-based devices. The remaining challenges include improving switching times and achieving local addressability of the responsive constituent. We envisage tackling these challenges by utilizing redox-responsive polymers which offer fast and reversible switching, and local addressability in combination with nanofabricated electrodes.

3.1 Introduction

Controlled manipulation and delivery of small amounts of liquids down to attoliters require sophisticated fluidic devices that offer smart actuation volume with full external fluid flow control. The availability of such microfluidic systems would enhance biomolecular separation, controlled transport of drugs, and micro- and nanoparticles and biomolecules on the micrometer or sub-micrometer length scales. Applications can also include power sources such as devices allowing for multi-stream laminar flow in fuel cells.^[1] Despite great progress in the development of nanofluidic devices,^[2-3] numerous challenges remain regarding addressable switches and actuators for fluid control. In this respect, smart materials composed of stimulus-responsive polymers are particularly promising candidates to tackle these challenges. Here we give a short account on the current status of this field and sketch some opportunities for future development.

Stimulus-responsive polymers undergo large and abrupt changes in their chemical and physical properties in response to small variations in various stimuli in their environment.^[4] Conventional stimuli include changes in temperature,^[5-10] pH^[11-16] ionic strength,^[17] electric^[18-19] and magnetic field,^[20] or a combination of these.^[21-22] For instance, the degree of stretching in polyelectrolyte brushes is strongly affected by changes in pH,^[23] ionic strength and the nature of the electrolyte in solution.^[24] Stimulus-responsive polymers have been intensively researched^[25-26] and a plethora of applications for such polymers has been identified and developed^[27-31] ranging from switching surface wetting and adhesion, to protective coatings that adapt to the environment, artificial muscles, sensors and drug delivery.^[32-34]

In the case of microfluidics, the responsiveness of polymers is especially apparent when they are densely grafted to the substrate, as shown schematically in Figure 3.1. Highly controlled preparation of polymer brushes in-situ is possible via controlled radical polymerization, for example, by employing surface-attached initiators.^[35] For their use as actuators in microfluidic systems, the reversible expansion and shrinking of such brushes offer great opportunities, which can be utilized as both passive and active fluid control elements to establish gate and valve functions and pumping elements. Active systems are defined as systems where the polymer reacts to a certain stimulus, e.g. irradiation with light or a change in pH, inducing, for example, a change in flow rate. Passive systems are defined as systems in which the polymer does not actively contribute to the flow mechanism; the desired effect is static, and a polymer coating that lowers the electro-osmotic flow constitutes a typical example.

Figure 3.1: Various stimulus-responsive polymer systems can be placed in a channel to control microfluidic flow, by, for example, swelling and collapsing a brush or hydrogel coatings. Stimuli that are commonly used in fluidic devices encompass irradiation with light and changes in temperature, pH and of the redox state.

Fluid control and robust performance have been demonstrated in microfluidic devices by several groups.^[36-38] However, the use of external stimuli which addresses the grafts at the ensemble level as opposed to the local trigger, is significantly limited in precise applications on small length scales, in particular if complex or synchronized actuation is needed. Temperature and pH variations are among such stimuli, but even the use of light has spatial limitations due to diffraction limits. Additionally, switching times are in the order of tens of seconds in the case of thermoresponsive polymers,^[39] up to even minutes in the case of photoresponsive polymers.^[40] These characteristics obviously pose limitations in applications for micro-/nanofluidic actuation. Finally, pH or ionic strength as stimuli can only be applied directly via delivery of sample liquids; i.e. no controlled local response is possible when using these systems. In short, an independent external switching mechanism on a small scale represents a severe bottleneck for microfluidic actuators and needs to be addressed if further progress is to be made in micro and nanofluidics.

It is generally considered, that an ideal microfluidic component has several important characteristics. Firstly, the response time of the component should be short, that is, in the microsecond range. Secondly, it should be at least as chemically resistant as the channels of the device. This is a property that is unlikely to be realized in the case of

organic materials; hence such systems will not be considered in this review. Thirdly it should be straightforward to fabricate the device including the treatment with stimulus-responsive polymers, for example, under ambient conditions. Finally, the device should be able to operate using a broad range of different liquids.

These challenges could be overcome if an electrical potential could be employed as the stimulus, for instance, using redox active polymers such as poly(ferrocenylsilane)s. One option is offered by organometallic polymers, including poly(ferrocenylsilane)s (PFS). PFSs are redox-active polymers with alternating silane and ferrocene moieties in the main chain,^[41-43] which can be covalently attached to a surface^[31] or used as a cationic or anionic coating.^[44] Electrochemical stimuli induce reversible changes of individual PFS chains by adjusting the redox state of PFS in an electrolyte solution. At surface grafted PFS brushes the reversible height change due to swelling upon electrochemical oxidation was observed.^[45-46] In addition adhesive properties were switched electrochemically.^[47] Hence, PFS polymer films represent promising candidates as active and passive elements that can be switched electrochemically within designed microfluidic devices. In general, the potential of redox polymers as active elements in microfluidic devices has yet to be explored despite promising results that have been published on the computational design of mixers and pumps for microfluidic systems.^[48] A key advantage of electrochemically applied redox stimuli over classic stimuli (changes in temperature, pH, ionic strength) would be their utility for fast and reversible switching between the oxidized and the reduced states. Critically, redox stimuli can be applied locally and, due to the wide availability of nanofabricated electrodes^[49-50] with reported widths down to 2 nm,^[51] integration into microfluidic devices becomes feasible, thereby allowing the development of sophisticated nanoscale actuators.

In this chapter we give an overview of selected examples from the literature reporting on the use of stimulus-responsive polymers as active or passive components for fluid control in glass microfluidic devices. However, in microfluidics, poly(dimethylsiloxane) (PDMS) is widely used as the main structural material due to easy processing and good material characteristics,^[52-54] but polymer coatings have also been applied to other microchannel materials, e.g. poly(methyl methacrylate),^[55-56] silicon,^[57] poly(ethylene terephthalate),^[58] etc. Polymer-coated PDMS is used in electro-osmotic flow experiments,^[59-63] and in pH-responsive,^[64] thermoresponsive^[65-66] and photoresponsive^[67] devices. For a more in-depth review about polymers coated to PDMS please refer to the reviews by Zhou et al.^[68-69] and Wong and Ho.^[70]

However versatile, PDMS has several drawbacks compared to glass, mainly in terms of solvent compatibility^[71] and wettability. Also leaching of uncured PDMS into the fluid is reported and influences biological compatibility.^[72-73] Furthermore, the elasticity of PDMS restricts the amount of pressure that can be used in these devices.

Because of these drawbacks, this review focuses mainly on polymers “grafted to” or “grafted from” microchannels in glass or fused silica.

3.2 pH-responsive polymers in fluidics

One of the first applications of hydrogels as valves in microfluidic channels was described by Beebe et al.^[74], who used the swelling and shrinking properties of a pH-responsive hydrogel to create a valve.^[75] 2-Hydroxyethyl methacrylate in acrylic acid (4:1 ratio) and ethylene glycol dimethacrylate (1 wt%) were photopolymerized using an inkjet-printed mask and Irgacure 651 as a photoinitiator (3 wt%). The hydrogel valve was placed on a membrane such that the swelling gel sealed the fluidic channel. A second design implemented the swelling gel as a throttle valve by using an array of gel columns.^[74] Already Beebe’s design showed the versatility required for the fabrication of microfluidic devices: in-situ fabrication of valves using photolithography gives the option for rapid prototyping, as the authors have shown in their work. Their fastest switching time was found to be 16 s for a full cycle and required a pH change in the channel from below 4 to above 9. In later work,^[75] this requirement was eliminated by separating the fluidic pathway from the pH regulation. The gel used in this study was separated from the fluidics by a poly(dimethylsilane) (PDMS) membrane. A response time of 24 s (under the assumption that the opening time was as fast as the closing time) was realized, which effectively removed the need for the pneumatics normally used in membrane valves by using a pH-responsive gel and a solution of a certain pH. However, the use of a PDMS membrane severely limits the choice of organic solvents. Furthermore, the response time should be further improved in order to qualify for high-throughput microfluidics.

A different pH-responsive system was devised by Salim et al.^[76] In order to investigate the influence of a pH responsive polymer on the electro-osmotic flow (EOF), they plasma-polymerized three different polymers, namely poly(tetraglyme), poly(acrylic acid) (PAA) and poly(allylamine), and coated the molecules to a microchannel wall. Polymerization was done *in-situ*.^[77] When performing EOF measurements, they discovered that the magnitude and the sign of the electro-osmotic mobility depend on the pH, enabling optimization of capillary electrophoresis when working with solutions of various pH. In a follow-up experiment, the effect of protein adsorption on the electro-osmotic mobility was also investigated and the mobility was found to be dependent on the concentration of the proteins used, namely fibrinogen and lysozyme, except for the poly(tetraglyme) coated channels, indicating that this is a suitable coating for protein-separation experiments. Covalently binding coatings to the channel greatly improves the number of times that the device can be used without the need for re-coating, compared to physically adsorbed layers. By using these coatings and tuning the pH the electro-osmotic mobility can be controlled. However, the chip used was a PTFE/glass device, clamped together by the set-up, which can become problematic in applications where high fluidic pressure is required.

3.3 Photoresponsive components

pH-responsive devices lack high speed and require addressability through a channel. By using light as an actuator, the in-channel requirement can be removed. Walsh et al.^[78] synthesized poly(spiropyran-co-divinylbenzene) in a poly(tetrafluoroethylene)-coated fused silica capillary with a diameter of 100 μm . When irradiated with ultraviolet (UV) light, the C-O bond in the spiropyran moiety breaks, converting the colorless spiropyran to merocyanine. Irradiation with visible light converts the merocyanine back into the spiropyran.^[79]

The electro-osmotic flow was controlled using this property. Irradiating with UV light for 120 seconds reduced the electro-osmotic flow by 50%. After irradiating with visible light, the flow rate increased again. The increase in flow rate is from 75 nl min^{-1} to 150 nl min^{-1} . This is too low a flow rate for many uses, since flow rates in microfluidics are usually in the microliters per minute range. Combined with the low switching time of at least 2 minutes, using this system as a high-throughput, fast microfluidic component will be challenging.

3.4 Thermoresponsive constituents

Arora et al.^[80] were inspired by an earthworm to fabricate a thermoresponsive device to displace a glass bead. Their active element was a poly(N-isopropylacrylamide) (PNIPAM) hydrogel. This gel has a lower critical solution temperature (LCST) of 32°C in aqueous environment, which means that it shrinks above this temperature. The collapse of the polymer results in a change in various properties, like friction, adhesion and solvability.^[81-82] This change can be exploited in microfluidic systems both as a valve and as a pump.

Using Peltier elements, the gel was locally heated and cooled, and the accompanying expanding/shrinking cycle resulted in the directed motion of the gel through a microchannel, the process is schematically displayed in Figure 3.2. However, one cycle takes 40 minutes to complete. Wall slippage of the gel was also investigated and a rough channel wall was found to be beneficial for gel motion. In later work, the gel was used to transport a glass bead in a channel with a radius of 500 μm , which attained speeds of up to 15 $\mu\text{m s}^{-1}$.^[83] The drawback of most temperature responsive systems, namely, the speed of actuation, is relevant here as well, as a full heating cooling cycle that was reported took 14 minutes. On the other hand, the low voltage used for the heating/cooling Peltier elements (1.5V) means that the device can be powered by a battery.

Figure 3.2: Schematic of motion of PNIPAM hydrogel by controlled heating and cooling, by Arora et al.^[80] By heating the channel at end 1, the polymer shrinks, contracting away from the heat source towards end 2. By subsequent cooling at end 2, the polymer swells again, away from both 'ends'. When the swelling is complete, the polymer is displaced. Copyright (c) 2009 Wiley Periodicals, Inc.

Richter et al.^[84] used a PNIPAM hydrogel as a 'normally closed' valve. To place the gel inside the channel, they photopolymerized PNIPAM in-situ. Locally heating the hydrogel using platinum resistors, open/close cycles of around 14 seconds could be realized. However, the maximum pressure that the valve could sustain was not investigated. In later work, they used PDMS instead of Pyrex glass as the cover layer.^[85] This was done to create an elastic membrane that could be either displaced by the PNIPAM gel in a separate area or used as a counter-pressure mechanism. The first design used the gel as an actuator to close a channel by pressure generated by the swelling of the gel, pressing on the PDMS membrane, effectively blocking the flow, as shown in Figure 3.3. The second design used the swelling and shrinking effects to pump the liquid through the hydrogel itself (since swelling adsorbs liquid) and by shrinking the liquid is expelled. The PDMS cover slip ensured directed motion due to elastic deformation. Using peristaltic pumping, a flow rate of $0.54 \mu\text{L min}^{-1}$ was obtained, furthermore, in pulsatile operation this could be increased to $2.8 \mu\text{L min}^{-1}$. However, pumping 'through' a hydrogel greatly depends on the type of liquid used, and these flow rates are only valid for deionized water.

Figure 3.3: Displacement pumps as fabricated by Richter et al.^[85] By controlled swelling and shrinking of the hydrogel actuator, the elastic membrane presses down on the pump chamber and flow can be stopped. By tuning the heating cycles with the heating resistors this setup is also used as a pump. Reproduced from Ref. ^[85] with permission from The Royal Society of Chemistry.

Using Peltier elements to actuate PNIPAM was also done by Yu et al.^[86] A PNIPAM gel was also used as valve. Since glass is a poor thermal conductor, actuation of the gel was done by integrated Peltier elements. Cavities were etched into the glass to incorporate the thermoelectrical elements, reducing the distance between the actuator and the gel. High-frequency experiments showed that one open/close cycle of the valve took 12 s. The closed valve maintained up to 1.38 MPa of fluid pressure without showing signs of damage or leakage.

In later work, by co-polymerizing the PNIPAM gel with N-ethylacrylamide (NEAM)^[87] a tunable LCST between 32 and 72°C was realized. A higher LCST means that only a heater is required, since the cooling cycle will happen once the gel releases its thermal energy to the surrounding environment. The optimum LCST was found to be 45°C, at equal NIPAM and NEAM molar ratios. A cycle time of 3-4 seconds was observed. The closed valve maintained operations up to 18.0 MPa. By using the integrated Peltier elements, the response time is lower than the photoresponsive or pH-responsive devices discussed above, but it is still far from microseconds.

Instead of using the swelling/shrinking of the PNIPAM around the LCST, Londe et al.^[88-89] used the hydrophobic/hydrophilic switch of the polymer to fabricate a valve. Above the LCST, PNIPAM is hydrophobic, whereas below it is hydrophilic. To coat the microchannel, 40 bilayers of poly(allylamine hydrochloride) (PAH) and silica nanoparticles were first deposited on the surface and annealed at 400°C for 2 hours, thereby increasing the roughness of the surface. On top of this surface, two bilayers of PAH and PAA were deposited, with PAA as the topmost layer and used as an anchor for the PNIPAM initiator. After NIPAM polymerization, the switching effect was further increased by chemical vapor deposition of a (1H,1H,2H,2H-perfluorooctyl) silane on the

polymer. The whole process results in a dramatic change in the water contact angle both above and below the LCST: from 14° at room temperature to 122° at 70°C . Using a dye, the valve was studied qualitatively and found to be working as expected, as can be seen in Figure 3.4. Unfortunately, neither the cycle time nor the time needed to heat and cool the sample was reported, therefore little can be said about the timescale on which this process operates. Furthermore the maximum pressure the valve can sustain was not characterized. However, the proof of principle works and shows promise of good chemical resistance due to the fluorinated silane as a top layer.

Figure 3.4: Closed (a and b) and open valve (c and d), by Londe et al.^[88] In the four different images the inlet is at the bottom of the image, in the left arm the valve is placed. The right column shows the fluid after reaching the valve (b and d). In the bottom-right image (d) the valve is open and the liquid can pass, the top-right image (c) shows the valve in the closed state and the liquid is blocked. Reprinted from ^[88], Copyright 2008, with permission from Elsevier.

A similar experiment to halt capillary flow was performed by Saitoh et al.^[90], their approach combined the use of Peltier elements with PNIPAM. Instead of optical inspection, spermine concentration measurements in saliva were used, using a two-junction method. At the first junction, the spermine was mixed with nickel(II) chloride for complex formation, whereas at the second junction a fluorescent marker, o-phthalaldehyde, was released, resulting in fluorescence. However, in this work, the authors did not specify the timescale on which these processes take place. Nevertheless, the experiments show that the idea of using PNIPAM as a valve has merit even when the PNIPAM is uncoated.

In order to control the flow rate in capillaries via temperature, Idota et al.^[91] coated microcapillaries with radii between $50\ \mu\text{m}$ and $500\ \mu\text{m}$ with a PNIPAM brush. Capillary height measurements were performed, revealing a change in capillary height as function of temperature, from this the contact angle was calculated. The contact angle changed from 58° at 10°C to 81° at 40°C in the case of a $300\ \mu\text{m}$ capillary. Changing the inner diameter of the channels led to no significant change in contact angle, if the temperature

remained constant. To confirm control of flow by temperature, two capillaries were placed in parallel, one coated with PNIPAM and one uncoated. Water was pumped in at 10 MPa from a single inlet. Below the LCST, no flow was measured through the coated capillary. However, above the LCST the flow rate was nearly equivalent to the rate observed in the uncoated channel. The cycle time of this device was 30 seconds. The high pressure this valve can sustain indicates that it is an excellent candidate for thermoresponsive release in microchannels, perhaps combined with exothermic catalysis.

Most thermoresponsive systems use PNIPAM as the active element. In an attempt to improve the response time of a thermoresponsive valve, Stoeber et al.^[92] used the triblock copolymer Pluronic F127 (poly(ethylene oxide)₁₀₆-poly(propylene oxide)₇₀-poly(ethylene oxide)₁₀₆) with on-chip integrated heaters to exploit the gel formation characteristics of the polymer as a valve. The polymer was dissolved in water (15 wt%) and transported through the channel. When locally heated with the integrated electrodes, fabricated in the channels and coated with a protective layer, the polymer formed a gel and blocked the channel. Results showed an open/close cycle of 33 ms. (The cycle time could actually be less, but the time resolution during these experiments was limited by the optical setup used, i.e. the video capture rate.) The fast response times were probably a result of the integrated electrode design. However, using this technique requires the polymer to be dissolved in the liquid, which limits the solvents that can be used. It also limits the use of other molecules dissolved in the liquid. Nevertheless, this is a good candidate if a pH-responsive device is not compatible with the system one wishes to employ.

In conclusion, from the properties of thermoresponsive polymers, swelling and shrinking are mostly used in microfluidic devices. Thermoresponsive polymers were reported as both valves and pumps. Characterization of the said valves was often found lacking in terms of the timescale of operation and pressure limitations, other than the proof of principle of the device little characterization was performed. However, for many systems the response time of the device turned out to be the bottleneck. Integrated electrodes are promising candidates to speed up these devices.

3.5 Passive elements

Passive systems are defined as systems in which the polymer does not *actively* contribute to the flow mechanism; the desired effect is static, and a polymer coating that lowers the electro-osmotic flow constitutes a typical example. Various types of devices have been reported, for example, pumps, valves, and coatings that modify the electro-osmotic flow for both flow reduction and DNA separation.

For instance, in order to fabricate a field-free electro-osmotic pump, Joo et al.^[93] used poly(diallyldimethylammonium chloride) and poly(styrene sulfonate) as the cationic and anodic coatings, respectively. By coating two arms of a Y-shaped microfluidic channel

with coatings of opposite polarity, a field-free flow could be created in the third arm, when the channel was filled with 10 mM phosphate buffer at pH 7.0, as shown schematically in Figure 3.5. Using this technique, field-free gated injection and on-chip field-free sorting were accomplished with sorting rates of up to 120 samples per minute. However, this is far below other systems that go up to 100 samples per second, but it shows a proof of principle of the novel idea of using a momentum-driven electro-osmotic pump.

Figure 3.5: Field-free flow generation, by Joo et al.^[93] Two arms are coated with polymers with different charge. Depending on the polarity of the electric field from one arm to the other, flow is directed inward or outward (blue arrows) in the third channel. Reprinted from ^[93], Copyright 2007, with permission from Elsevier.

For fabricating a valve, Kirby et al.^[94] photopolymerized a polymer plug, that could be pressed, using pneumatics, against a valve seat in a channel. The plug blocked the channel, stopping the flow. The monomer was 2,2,3,4,4,4-hexafluorobutyl acrylate cross-linked with 1,3-butanediol diacrylate. Using this polymer monolith, they could close a 65 μm diameter cylindrical microchannel with pressures up to 7.0 MPa, limited only by the external materials or the glass substrate. The leakage was measured and found to be negligible. Further studies showed the response time to be 33 ms.^[95] Interestingly, this response time is similar to that reported by Stoeber et al.^[92] giving the impression that this response time is perhaps also limited by the frame rate of the optical setup (30 frames per second). Using the polymer plug, a one-directional valve was fabricated by Hasselbrink et al.^[95], using the flow pressure instead of the external pneumatics to close the valve in one direction, but leaving it open in the other. This type of component should show high chemical resistance due to presence of fluorinated alkanes and would prevent flow going towards the inlet. One drawback could be the sliding of the polymer plug: the lifetime of the plug has yet to be investigated.

Several groups have done work on reducing the flow internally. For example, in a rectangular microchannel, the four walls were individually modified with poly(neopentylmethacrylamide-co-N-4-(trimethylsilyl)phenylmethacrylamide), by Sultana et al.^[96] This hydrophobic coating resulted in various shapes of the air/water interface in the channel for the four possibilities. A tapered shape was seen when a single wall was coated, and a saddle-like shape was seen when opposite walls were coated. The flow rate was found to be dependent on the surface modification, but the flow rates

dropped as more walls were coated. Since these authors used a three layer system, they could achieve all possible combinations of hydrophilic and hydrophobic walls. However, the substrates were bonded using adhesives, which limited the solvent compatibility of the system. A different approach was done by Lanotte et al.^[97] They showed that grafting a poly(hydroxyethylmethacrylate) brush from a fused silica surface reduced the flow rate, more than would have been expected of the reduced channel diameter. The maximum velocity in the channel of the pressure-driven flow decreased by 35%. The authors explain their results with the aid of molecular simulations and conclude that the decrease in velocity is due to the brushes stretching and recoiling, resulting in a net backflow near the channel wall. However, statically reducing the flow rate is of little use in microfluidic components and shall be used, as the authors point out, as a prelude to studying blood flow in the microvascular system.

To control the electro-osmotic mobility as a function of surfactant concentration in their channels, Mora et al.^[98] coated fused silica capillaries with PDMS. Coated capillaries were used instead of PDMS microchannels to readily combine the channel with a regular capillary electrophoresis setup. Using sodium dodecyl sulfate, a non-linear increase in mobility was observed when increasing the concentration; this was ascribed to the change in zeta potential due to the electrolytes adsorbing to the PDMS surface. The result is shown in Figure 3.6.

Figure 3.6: Effect of adding sodium dodecyl sulfate (SDS) to the electrolyte solution in bare (open rectangles) or PDMS-coated (closed circles) capillaries. From Mora et al.^[98] Coated capillaries show a clear effect of the surfactant concentration on the electro-osmotic mobility. Reprinted with permission from ^[98]. Copyright 2007 American Chemical Society.

To investigate the effect of gold nanoparticles on electrophoresis, Pumera et al.^[99] coated a glass capillary with poly(diallyldimethylammonium chloride) and flushed the channel with a citrate-stabilized gold nanoparticle solution for 30 minutes. When measuring electropherograms of the coated and uncoated channels, they found that, for aminophenols, the resolution increased dramatically. However, on the downside, the

analysis time increased from 1.5 minutes to 2.5 minutes, due to the longer retention time of the analyte. As a control experiment, they also performed electrophoresis on channels without the gold nanoparticles, but found that no analytes reached the detector. However, only aminophenols were investigated, and it is not certain that this modification will increase the resolution of measurements on other molecules.

Kohlheyer et al.^[100] fabricated a free-flow electrophoresis device using poly(acrylamide) as an ion permeable membrane as a prelude for a device with a pH gradient. An *in-situ* photopolymerized membrane was used as an electrical connection between the electrodes and the sample channel. At the same time the membrane resisted hydrodynamic flow, ensuring that the channel is leak free. In later work, these authors realized a pH gradient across the channel using pre-separated ampholytes.^[101] The linearity of the pH gradient was confirmed using IEF markers ranging from pH 4 to 10, and the theoretic separation limit $\Delta(pI)$ was 0.23. The in-channel separation as well as the experiment without applied potential are shown in Figure 3.7. The gradient can be used for microreactors in combination with pH-sensitive reactions.

Figure 3.7: Free-flow isoelectric focusing; Kohlheyer et al.^[101] Top: device with no potential applied. Bottom: device with 150V applied. The in-channel separation of the markers from pH 4 to pH 10 can be clearly seen. Reprinted with permission from ^[101]. Copyright 2007 American Chemical Society.

Due to their diversity, polymer coatings are also used in biological applications. Wen et al.^[102], photopolymerized 3-(trimethoxysilyl)propyl methacrylate *in situ* as a solid state filter for DNA purification. Using a protein catching step, they achieved an extraction efficiency of 69% when using whole blood. When this device is used, a 2-propanol washing step when combining DNA capture with Polymerase Chain Reaction (PCR) to

remove unwanted proteins could be omitted. However, the question of the long-term stability of the filtering device was not addressed.

A different polymer for PCR products was used by Toriello et al.^[103], who copolymerized linear poly(acrylamide) with a 5' acrydite modified oligonucleotide as the capture element for nanoliter amounts of PCR products. A capture efficiency of 100% was reported. The captured products were released in a capillary electrophoresis (CE) channel for subsequent size-based separation. The proposed injection scheme, displayed in Figure 3.8, improved the signal strength a hundredfold.

Figure 3.8: The capture, purification and direct CE separation device from Toriello et al.^[103] The four-layer glass-PDMS-glass-glass device contains a PDMS pump (b), a capture region (c), separation channel and detection region. Each cycle pumps 10 nL of liquid to the hold chamber. Reprinted with permission from ^[103]. Copyright 2007 American Chemical Society.

Several different coatings for DNA separation by electrophoresis in fused-silica capillaries were investigated by Doherty et al.^[104] The polymer coatings tested were poly(acrylamide), poly(*N,N*-dimethylacrylamide), poly(*N,N*-diethylacrylamide) and *N,N*-diethylacrylamide/*N,N*-dimethylacrylamide random copolymers. The results showed that polymers of above 15000 monomer units reduced the electro-osmotic mobility significantly. Also, the coatings should be as hydrophilic as possible so that the electrical double layer is pierced.

Costantini et al.^[105] grew a 2-hydroxyethylmethacrylate-tetraethylene glycol dimethacrylate gel on the channel wall to immobilize silver and palladium nanoparticles in a microreactor. This was done to enhance the catalytic activities of the metal nanoparticles due to the increased specific surface area of a microchannel compared to conventional reactors. After reaction with succinic anhydride in dry pyridine, the channels were flushed with a 0.05 M silver or palladium nitrite solution in a 3:1 DMSO-water

mixture, resulting in 20 ± 5 and 30 ± 3 nm particles for Ag and Pd, respectively. In other work, poly(methacrylic acid) brushes were grown to study the immobilization of lipase using EDC-NHS chemistry.^[106] When studying the activity of the enzyme using 4-nitrophenol acetate they found that the reactivity of the enzyme did not change after immobilization on the surface. In recent work, poly(2-hydroxyethyl methacrylate) brushes were used to link glucose-oxidase and horseradish peroxidase to the channel surface. Using an enzymatic cascade reaction, they could detect glucose in blood within 20 s, with the limit of detection and quantification being 60 μ M and 200 μ M, respectively.^[107] The authors reported high stability and reusability of their enzymes. The papers discussed show that stimulus-responsive polymers in microfluidics are a promising technology for PCR separation, enzyme activity studies and biological applications in general.

A summary of all discussed research, both active and passive, is given in Table 3.1.

Table 3.1: Summary of polymer systems

Monomer	Polymer	Initiator	Type of pol.	Deposition	Response	Ref
2-hydroxyethyl methacrylate/acrylic acid	gel	Irigature	photo-pol.	photomask	pH	74
tetraglyme, acrylic acid, allylamine	film	n/a	plasma-pol.	vapor phase	pH	76
spiroiran-co-divinylbenzene	copolymer film	2,2-azobisisobutyronitrile	60°C, 5 days	adsorption from liquid	light	78
N-isopropylacrylamide	gel	potassium persulfate and TEMED	radical pol.	liquid	thermal	80
N-isopropylacrylamide	gel	2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone	photo-pol.	photomask	thermal	85
N-isopropylacrylamide	gel	4,4'-azobis(4-cyanovaleic acid)	photo-pol.	3-(trimethoxysilyl)propyl methacrylate	thermal	86
N-isopropylacrylamide/N-ethylacrylamide	gel copolymer	4,4'-azobis(4-cyanovaleic acid)	photo-pol.	3-(trimethoxysilyl)propyl methacrylate	thermal	87
N-isopropylacrylamide	film	2,2'-azobis(2-methylpropanamide) dichloride	65°C	adsorption from liquid	thermal	88
N-isopropylacrylamide	film	ammonium persulfate and TEMED	radical pol.	allyltrimethoxysilane	thermal	90
N-isopropylacrylamide	brush	4,4'-azobis(4-cyanovaleic acid)	surface-immobilized radical pol. and electron-beam irradiated pol.	3-aminopropyltriethoxy silane	thermal	91
Pluronic F127	triblock copolymer	n/a	n/a	in-situ gelation	thermal	92
diallyldimethylammonium chloride styrene sulfonate	film	n/a	n/a	layer-by-layer	passive	93
2,2,3,4,4,4-hexafluorobutyl acrylate	solid	2,2-azobisisobutyronitrile	photo-pol.	photomask	passive	94
neopentylmethacrylamide-co-N-4-(trimethylsilyl)phenylmethacrylamide	copolymer	2,2-azobisisobutyronitrile	free radical pol.	Langmuir-Blodgett	passive	96

Monomer	Polymer state	Initiator	Type of pol.	Deposition	Response	Ref
2-hydroxyethylmethacrylate	brush	2-bromo-2-methylpropionyl bromide	atom transfer radical pol.	3-aminopropyltriethoxy silane	passive	97
dimethylsiloxane	film	n/a	thermal crosslinking	adsorption from liquid	passive	98
diallyldimethylammonium chloride	film	n/a	n/a	adsorption from liquid	passive	99
acrylamide	membrane	2,2-dimethoxy-2-phenylacetophenone	photo-pol.	3-(trimethoxysilyl) propyl methacrylate	passive	100
3-(trimethoxysilyl)propyl methacrylate	solid	Irgacure	photo-pol.	photomask	passive	102
acrylamide-co-acrydite modified oligonucleotide	gel	ammonium persulfate and TEMED	-	-	passive	103
acrylamide, N,N-dimethylacrylamide, N,N-diethylacrylamide, N,N-diethylacrylamide/N,N-dimethylacrylamide	(co)-polymer	ammonium persulfate and TEMED	50°C	adsorption from liquid	passive	104
2-hydroxyethyl methacrylate-tetraethylene glycol dimethacrylate	gel	2,2'-bipyridyl	atom transfer radical pol.	3-(trichlorosilylpropyl) 2-bromo-2-methylpropionate	passive	105
methacrylic acid	brush	n/a	photo-pol.	EDC-NHS	passive	106
2-hydroxyethyl methacrylate	brush	2,2'-bipyridyl	atom transfer radical pol.	3-(trichlorosilylpropyl) 2-bromo-2-methylpropionate	passive	107

Abbreviations: N,N,N',N'-tetramethylethylenediamine (TEMED), Polymerization (pol.)

3.6 Conclusions

A variety of stimulus responsive polymers have been used as active or passive elements for fluid control in glass- and PDMS based microfluidic devices. Usually, the response of these polymers is triggered by external stimuli at the medium (ensemble) level, including variations in temperature, solvent composition, pH or illumination with light. Although fluid control has been demonstrated in microfluidic devices in a number of publications, a severe drawback of external stimuli consists of locally controlling responsive elements of functional structures on small scales, ideally on the nanometer length scale. In addition, switching times of seconds to several minutes severely hamper usability for highly controlled and advanced fluid delivery. The latter is especially so for thermally controlled systems. Nevertheless, these systems show that they can withstand high fluidic pressures which means that robust components can be fabricated from thermoresponsive polymers, mainly when timescales are not of importance. However, for biological applications, the operating temperature window is small, and mostly passive systems were reported in this regard, where covalent binding of the polymer coating to the channel wall has preference over physical adsorption to prevent solvent contamination. Applying stimulus through the solvent, as in the case of pH-responsive systems, should be avoided to be able to use a broad range of solvents and solutes. However, if the solvent is compatible, pH-responsive systems are a strong candidate for microfluidic components. We propose to tackle these challenges, namely fast switching, solvent compatibility and biological applicability, by utilizing redox-responsive polymers which offer fast, reversible switching and local addressability in combination with nanofabricated electrodes. For example, a poly(ferrocenylsilane) based hydrogel cross-linked with PNIPAM can be reduced and oxidized isothermally, collapsing and swelling the gel accordingly.^[22] Microfluidic devices have not been constructed from poly(ferrocenylsilane)s. When applied to a microchannel wall a peristaltic pump could be created. Alternatively, a redox-active polymer could be used to change the hydrophobicity in the channel,^[108] thereby creating a capillary valve.

3.7 References

- [1] E. Choban, *J. Power Sources* **2004**, 128, 54-60 | 10.1016/j.jpowsour.2003.11.052
- [2] G. M. Whitesides, *Nature* **2006**, 442, 368-73 | 10.1038/nature05058
- [3] E. Kumacheva, P. Garstecki, *Microfluidic Reactors for Polymer Particles*. Wiley: **2011**; p 236.
- [4] J. M. Swann, A. J. Ryan, *Polym. Int.* **2009**, 58, 285-289 | 10.1002/pi.2536
- [5] D. Kuckling, H.-J. P. Adler, K.-F. Arndt, L. Ling, W. D. Habicher, *Macromol. Chem. Phys.* **2000**, 201, 273-280 | 10.1002/(SICI)1521-3935(20000201)201:2<273::AID-MACP273>3.0.CO;2-E
- [6] E. M. Benetti, S. Zapotoczny, G. J. Vancso, *Adv. Mater.* **2007**, 19, 268-271 | 10.1002/adma.200601554
- [7] D. Kuckling, M. E. Harmon, C. W. Frank, *Macromolecules* **2002**, 35, 6377-6383 | 10.1021/ma0203041
- [8] A. M. Jonas, K. Glinel, R. Oren, B. Nysten, W. T. S. Huck, *Macromolecules* **2007**, 40, 4403-4405 | 10.1021/ma070897l
- [9] M. N. Moghadam, V. Kolesov, A. Vogel, H.-A. Klok, D. P. Pioletti, *Biomaterials* **2014**, 35, 450-5 | 10.1016/j.biomaterials.2013.09.065
- [10] M. Szuwarzyński, L. Zaraska, G. D. Sulka, S. Zapotoczny, *Chem. Mater.* **2013**, 25, 514-520 | 10.1021/cm303930y
- [11] D. Kuckling, C. D. Vo, S. E. Wohlrab, *Langmuir* **2002**, 18 (11), 4263-4269 | 10.1021/la015758q
- [12] A. J. Chung, M. F. Rubner, *Langmuir* **2002**, 18, 1176-1183 | 10.1021/la010873m
- [13] S. Y. Yang, M. F. Rubner, *J. Am. Chem. Soc.* **2002**, 124, 2100-1 | 10.1021/ja017681y
- [14] J. A. Hiller, M. F. Rubner, *Macromolecules* **2003**, 36, 4078-4083 | 10.1021/ma025837o
- [15] H. Lee, C. Sample, R. E. Cohen, M. F. Rubner, *ACS Macro Letters* **2013**, 2, 924-927 | 10.1021/mz400398s
- [16] E. M. Benetti, H. J. Chung, G. J. Vancso, *Macromol. Rapid Commun.* **2009**, 30 (6), 411-7 | 10.1002/marc.200800628
- [17] H. Li, F. Lai, R. Luo, *Langmuir* **2009**, 25, 13142-50 | 10.1021/la901833m
- [18] Y. Osada, H. Okuzaki, H. Hori, *Nature* **1992**, 355, 242-244 | 10.1038/355242a0
- [19] M. A. Hempenius, C. Cirri, F. Lo Savio, J. Song, G. J. Vancso, *Macromol. Rapid Commun.* **2010**, 31 (9-10), 772-783 | 10.1002/marc.200900908
- [20] G. Filipcsei, I. Csetneki, A. Szilágyi, M. Zrínyi, Magnetic Field-Responsive Smart Polymer Composites. In *Oligomers Polymer Composites Molecular Imprinting*, Springer Berlin Heidelberg: 2007; Vol. 206, pp 137-189.
- [21] R. Cheng, F. Meng, C. Deng, H.-A. Klok, Z. Zhong, *Biomaterials* **2013**, 34, 3647-57 | 10.1016/j.biomaterials.2013.01.084
- [22] X. Sui, X. Feng, A. Di Luca, C. A. van Blitterswijk, L. Moroni, M. A. Hempenius, G. J. Vancso, *Polymer Chemistry* **2013**, 4, 337 | 10.1039/c2py20431b
- [23] M. Elzbieciak, S. Zapotoczny, P. Nowak, R. Krastev, M. Nowakowska, P. Warszyński, *Langmuir* **2009**, 25, 3255-9 | 10.1021/la803988k
- [24] B. Yu, H. Hu, D. Wang, W. T. S. Huck, F. Zhou, W. Liu, *J. Mater. Chem.* **2009**, 19, 8129 | 10.1039/b910279e
- [25] F. Zhou, W. T. S. Huck, *PCCP* **2006**, 8 (33), 3815 | 10.1039/b606415a

- [26] D. Kuckling, A. Wycisk, *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *51*, 2980-2994 | 10.1002/pola.26696
- [27] Z. Nie, E. Kumacheva, *Nature materials* **2008**, *7*, 277-90 | 10.1038/nmat2109
- [28] S. Zapotoczny, *Methods in molecular biology* **2012**, *811*, 51-78 | 10.1007/978-1-61779-388-2_4
- [29] D. Kuckling, *Colloid. Polym. Sci.* **2009**, *287*, 881-891 | 10.1007/s00396-009-2060-x
- [30] C. de las Heras Alarcón, T. Farhan, V. L. Osborne, W. T. S. Huck, C. Alexander, *J. Mater. Chem.* **2005**, *15*, 2089 | 10.1039/B419142K
- [31] X. Sui, X. Feng, J. Song, M. a. Hempenius, G. J. Vancso, *J. Mater. Chem.* **2012**, *22*, 11261 | 10.1039/c2jm30599b
- [32] D. Jagadeesan, I. Nasimova, I. Gourevich, S. Starodubtsev, E. Kumacheva, *Macromol. Biosci.* **2011**, *11*, 889-96 | 10.1002/mabi.201100045
- [33] A. Rösler, G. W. Vandermeulen, H. A. Klok, *Adv. Drug Del. Rev.* **2001**, *53*, 95-108 | 10.1016/S0169-409X(01)00222-8
- [34] M. A. Cohen Stuart, W. T. S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov, S. Minko, *Nature materials* **2010**, *9*, 101-13 | 10.1038/nmat2614
- [35] R. Barbey, L. Lavanant, D. Paripovic, N. Schüwer, C. Sugnaux, S. Tugulu, H.-A. Klok, *Chem. Rev.* **2009**, *109*, 5437-527 | 10.1021/cr900045a
- [36] W. H. Grover, A. M. Skelley, C. N. Liu, E. T. Lagally, R. A. Mathies, *Sensors Actuators B: Chem.* **2003**, *89*, 315-323 | 10.1016/S0925-4005(02)00468-9
- [37] N.-T. Nguyen, X. Huang, T. K. Chuan, *J. Fluids Eng.* **2002**, *124*, 384 | 10.1115/1.1459075
- [38] P. Woias, *Sensors Actuators B: Chem.* **2005**, *105*, 28-38 | 10.1016/j.snb.2004.02.033
- [39] X. Sui, Q. Chen, M. A. Hempenius, G. J. Vancso, *Small* **2011**, *7*, 1440-7 | 10.1002/smll.201002229
- [40] C. L. Feng, Y. J. Zhang, J. Jin, Y. L. Song, L. Y. Xie, G. R. Qu, L. Jiang, D. B. Zhu, *Langmuir* **2001**, *17*, 4593-4597 | 10.1021/la010071r
- [41] Y. Ni, R. Rulkens, I. Manners, *J. Am. Chem. Soc.* **1996**, *118*, 4102-4114 | 10.1021/ja953805t
- [42] I. Manners, *Chem. Commun.* **1999**, 857-865 | 10.1039/a810043h
- [43] G. R. Whittell, M. D. Hager, U. S. Schubert, I. Manners, *Nature materials* **2011**, *10*, 176-88 | 10.1038/nmat2966
- [44] M. A. Hempenius, F. F. Brito, G. J. Vancso, *Macromolecules* **2003**, *36*, 6683-6688 | 10.1021/ma034432g
- [45] M. Péter, M. A. Hempenius, E. S. Kooij, T. A. Jenkins, S. J. Roser, W. Knoll, G. J. Vancso, *Langmuir* **2004**, *20*, 891-897 | 10.1021/la035656v
- [46] M. Péter, R. G. H. Lammertink, M. A. Hempenius, G. J. Vancso, *Langmuir* **2005**, *21*, 5115-23 | 10.1021/la0473409
- [47] X. Feng, B. D. Kieviet, J. Song, P. M. Schön, G. J. Vancso, *Appl. Surf. Sci.* **2014**, *292*, 107-110 | 10.1016/j.apsusc.2013.11.087
- [48] K. Kannappan, G. Bogle, J. Trivas-Sejdic, D. E. Williams, *Physical chemistry chemical physics : PCCP* **2011**, *13*, 5450-61 | 10.1039/c0cp02659j
- [49] C. G. Zoski, *Electroanalysis* **2002**, *14*, 1041-1051 | 10.1002/1521-4109(200208)14:15/16<1041::AID-ELAN1041>3.0.CO;2-8
- [50] R. W. Murray, *Chem. Rev.* **2008**, *108*, 2688-720 | 10.1021/cr068077e
- [51] R. B. Morris, D. J. Franta, H. S. White, **1987**, 3559-3564 | 10.1021/J100297a019

- [52] S. Xu, Z. Nie, M. Seo, P. Lewis, E. Kumacheva, H. A. Stone, P. Garstecki, D. B. Weibel, I. Gitlin, G. M. Whitesides, *Angewandte Chemie (International ed. in English)* **2005**, *44*, 724-8 | 10.1002/anie.200462226
- [53] D. Qin, Y. Xia, G. M. Whitesides, *Nature protocols* **2010**, *5*, 491-502 | 10.1038/nprot.2009.234
- [54] J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, O. J. A. Schueller, G. M. Whitesides, *Electrophoresis* **2000**, *21*, 27-40 | 10.1002/(SICI)1522-2683(20000101)21:1<27::AID-ELPS27>3.0.CO;2-C
- [55] S. L. Barker, D. Ross, M. J. Tarlov, M. Gaitan, L. E. Locascio, *Anal. Chem.* **2000**, *72*, 5925-9 | 10.1021/ac0008690
- [56] S. Barker, M. Tarlov, H. Canavan, J. Hickman, L. Locascio, *Anal. Chem.* **2000**, *72*, 4899-903 | 10.1021/ac000548o
- [57] J. P. DeRocher, P. Mao, J. Han, M. F. Rubner, R. E. Cohen, *Macromolecules* **2010**, *43*, 2430-2437 | 10.1021/ma902451s
- [58] W. Chen, T. J. McCarthy, *Macromolecules* **1997**, *30*, 78-86 | 10.1021/Ma961096d
- [59] A.-J. Wang, J.-J. Xu, H.-Y. Chen, *Journal of chromatography. A* **2006**, *1107*, 257-64 | 10.1016/j.chroma.2005.12.040
- [60] A. K. Price, K. M. Anderson, C. T. Culbertson, *Lab on a chip* **2009**, *9*, 2076-84 | 10.1039/b823465e
- [61] Q. L. Zhang, J. J. Xu, H. Z. Lian, X. Y. Li, H. Y. Chen, *Anal. Bioanal. Chem.* **2007**, *387* (8), 2699-704 | 10.1007/s00216-007-1173-7
- [62] Z. D. Kihm, E. M. Veen, J. D. Bergen-Hartigan, Y. Zhang, Y. Liu, *Anal. Sci.* **2012**, *28*, 183-6 | 10.2116/analsci.28.183
- [63] A. J. Wang, J. J. Xu, H. Y. Chen, *J. Chromatogr. A* **2007**, *1147* (1), 120-6 | 10.1016/j.chroma.2007.02.030
- [64] D. T. Eddington, R. H. Liu, J. S. Moore, D. J. Beebe, *Lab on a chip* **2001**, *1*, 96-9 | 10.1039/b108078d
- [65] S. Sugiura, W. Imano, T. Takagi, K. Sakai, T. Kanamori, *Biosens. Bioelectron.* **2009**, *24* (5), 1135-40 | 10.1016/j.bios.2008.06.038
- [66] L. K. Fiddes, H. K. C. Chan, B. Lau, E. Kumacheva, A. R. Wheeler, *Biomaterials* **2010**, *31*, 315-20 | 10.1016/j.biomaterials.2009.09.040
- [67] S. Sugiura, K. Sumaru, K. Ohi, K. Hiroki, T. Takagi, T. Kanamori, *Sensors and Actuators A: Physical* **2007**, *140*, 176-184 | 10.1016/j.sna.2007.06.024
- [68] J. Zhou, A. V. Ellis, N. H. Voelcker, *Electrophoresis* **2010**, *31*, 2-16 | 10.1002/elps.200900475
- [69] J. Zhou, D. A. Khodakov, A. V. Ellis, N. H. Voelcker, *Electrophoresis* **2012**, *33*, 89-104 | 10.1002/elps.201100482
- [70] I. Wong, C.-M. Ho, *Microfluid. Nanofluid.* **2009**, *7*, 291-306 | 10.1007/s10404-009-0443-4
- [71] J. N. Lee, C. Park, G. M. Whitesides, *Anal. Chem.* **2003**, *75*, 6544-54 | 10.1021/ac0346712
- [72] M. W. Toepke, D. J. Beebe, *Lab on a chip* **2006**, *6*, 1484-6 | 10.1039/b612140c
- [73] K. J. Regehr, M. Domenech, J. T. Koepsel, K. C. Carver, S. J. Ellison-Zelski, W. L. Murphy, L. A. Schuler, E. T. Alarid, D. J. Beebe, *Lab on a chip* **2009**, *9*, 2132-9 | 10.1039/b903043c
- [74] D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss, B.-h. Jo, *Nature* **2000**, *404*, 588-590 | 10.1038/35007047
- [75] R. H. Liu, Q. Yu, D. J. Beebe, *Journal of Microelectromechanical systems* **2002**, *11* (1), 45-53 | 10.1109/84.982862

- [76] M. Salim, P. C. Wright, S. L. McArthur, *Electrophoresis* **2009**, *30*, 1877-87 | 10.1002/elps.200800619
- [77] M. Salim, G. Mishra, G. J. S. Fowler, B. O'sullivan, P. C. Wright, S. L. McArthur, *Lab on a chip* **2007**, *7*, 523-5 | 10.1039/b615328c
- [78] Z. Walsh, S. Scarmagnani, F. Benito-López, S. Abele, F.-Q. Nie, C. Slater, R. Byrne, D. Diamond, B. Paull, M. Macka, *Sensors Actuators B: Chem.* **2010**, *148*, 569-576 | 10.1016/j.snb.2010.04.048
- [79] S. Wang, Y. Song, L. Jiang, *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* **2007**, *8* (1), 18-29 | 10.1016/j.jphotochemrev.2007.03.001
- [80] H. Arora, R. Malik, L. Yeghiazarian, C. Cohen, U. Wiesner, *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 5027-5033 | 10.1002/pola.23555
- [81] H. G. Schild, *Prog. Polym. Sci.* **1992**, *17*, 163-249 | 10.1016/0079-6700(92)90023-R
- [82] S. de Beer, E. Kutnyanszky, P. M. Schön, G. J. Vancso, M. H. Müser, *Nat. Commun.* **2014**, *5*, 3781 | 10.1038/ncomms4781
- [83] L. Yeghiazarian, S. Mahajan, C. Montemagno, C. Cohen, U. Wiesner, *Adv. Mater.* **2005**, *17*, 1869-1873 | 10.1002/adma.200401205
- [84] A. Richter, D. Kuckling, S. Howitz, T. Gehring, K. Arndt, *Journal of Microelectromechanical Systems* **2003**, *12*, 748-753 | 10.1109/JMEMS.2003.817898
- [85] A. Richter, S. Klatt, G. Paschew, C. Klenke, *Lab on a chip* **2009**, *9*, 613-8 | 10.1039/b810256b
- [86] C. Yu, S. Mutlu, P. Selvaganapathy, C. H. Mastrangelo, F. Svec, J. M. J. Fréchet, *Anal. Chem.* **2003**, *75* (8), 1958-1961 | 10.1021/ac026455j
- [87] Q. Luo, S. Mutlu, Y. B. Gianchandani, F. Svec, J. M. J. Fréchet, *Electrophoresis* **2003**, *24*, 3694-702 | 10.1002/elps.200305577
- [88] G. Londe, A. Chunder, A. Wesser, L. Zhai, H. Cho, *Sensors Actuators B: Chem.* **2008**, *132*, 431-438 | 10.1016/j.snb.2007.10.052
- [89] A. Chunder, K. Etcheverry, G. Londe, H. J. Cho, L. Zhai, *Colloids Surf. Physicochem. Eng. Aspects* **2009**, *333*, 187-193 | 10.1016/j.colsurfa.2008.09.044
- [90] T. Saitoh, N. Suzuki, T. Furuse, M. Hiraide, *Talanta* **2009**, *80*, 1012-5 | 10.1016/j.talanta.2009.07.001
- [91] N. Idota, a. Kikuchi, J. Kobayashi, K. Sakai, T. Okano, *Adv. Mater.* **2005**, *17*, 2723-2727 | 10.1002/adma.200402068
- [92] B. Stoeber, Z. Yang, D. Liepmann, S. J. Muller, *Journal of Microelectromechanical systems* **2005**, *14* (2), 207-213 | 10.1109/Jmems.2004.839330
- [93] S. Joo, T. D. Chung, H. C. Kim, *Sensors Actuators B: Chem.* **2007**, *123* (2), 1161-1168 | 10.1016/j.snb.2006.10.069
- [94] B. J. Kirby, T. J. Shepodd, E. F. Hasselbrink, *Journal of chromatography. A* **2002**, *979*, 147-154 | 10.1016/S0021-9673(02)01453-X
- [95] E. F. Hasselbrink, T. J. Shepodd, J. E. Rehm, *Anal. Chem.* **2002**, *74* (19), 4913-4918 | 10.1021/ac025761u
- [96] S. Sultana, J. Matsui, M. Mitsuishi, T. Miyashita, *Thin Solid Films* **2009**, *518* (2), 606-609 | 10.1016/j.tsf.2009.07.048
- [97] L. Lanotte, S. Guido, C. Misbah, P. Peyla, L. Bureau, *Langmuir* **2012**, *28* (38), 13758-64 | 10.1021/la302171a

- [98] M. F. Mora, C. E. Giacomelli, C. D. Garcia, *Anal. Chem.* **2007**, 79, 6675-81 | 10.1021/ac070953g
- [99] M. Pumera, J. Wang, E. Grushka, R. Polsky, *Anal. Chem.* **2001**, 73, 5625-8 | 10.1021/ac015589e
- [100] D. Kohlheyer, G. A. J. Besselink, S. Schlautmann, R. B. M. Schasfoort, *Lab on a chip* **2006**, 6, 374-80 | 10.1039/b514731j
- [101] D. Kohlheyer, J. C. T. Eijkel, S. Schlautmann, A. van den Berg, R. B. M. Schasfoort, *Anal. Chem.* **2007**, 79, 8190-8 | 10.1021/ac071419b
- [102] J. Wen, C. Guillo, J. P. Ferrance, J. P. Landers, *Anal. Chem.* **2007**, 79 (16), 6135-6142 | 10.1021/ac0703698
- [103] N. M. Toriello, C. N. Liu, R. G. Blazej, N. Thaitrong, R. A. Mathies, *Anal. Chem.* **2007**, 79 (22), 8549-8556 | 10.1021/ac0712547
- [104] E. A. S. Doherty, K. D. Berglund, B. A. Buchholz, I. V. Kourkine, T. M. Przybycien, R. D. Tilton, A. E. Barron, *Electrophoresis* **2002**, 23, 2766-76 | 10.1002/1522-2683(200208)23:16<2766::AID-ELPS2766>3.0.CO;2-S
- [105] F. Costantini, E. M. Benetti, R. M. Tiggelaar, H. J. G. E. Gardeniers, D. N. Reinhoudt, J. Huskens, G. J. Vancso, W. Verboom, *Chemistry - A European Journal* **2010**, 16, 12406-11 | 10.1002/chem.201000948
- [106] F. Costantini, E. M. Benetti, D. N. Reinhoudt, J. Huskens, G. J. Vancso, W. Verboom, *Lab on a chip* **2010**, 10, 3407-12 | 10.1039/c0lc00187b
- [107] F. Costantini, R. Tiggelaar, S. Sennato, F. Mura, S. Schlautmann, F. Bordi, H. Gardeniers, C. Manetti, *The Analyst* **2013**, 138, 5019-24 | 10.1039/c3an00806a
- [108] J. Isaksson, C. Tengstedt, M. Fahlman, N. Robinson, M. Berggren, *Adv. Mater.* **2004**, 16, 316-320 | 10.1002/adma.200306131

In-line sensing of sodium ascorbate using a poly(ferrocenylsilane)-coated microfluidic device

Poly(ferrocenylsilane) (PFS) is a redox-active polymer that can be utilized in a variety of applications, such as sensing, nanoparticle foundries or dual-responsive hydrogels, due to the fact that it can be partially or completely oxidized, reversibly, by (electro)chemical means. In this chapter, PFS is tethered to a cysteamine-modified gold-coated microchannel electrode via amine alkylation, following a recent published procedure for conventional macro-sized electrodes. The PFS-coated microfluidic device is successfully employed as an electrochemical sensor for sodium L-ascorbate, or vitamin C, with a detection limit of 0.5 mM in aqueous electrolyte-solution. The fabrication of the device, that comprises gold-coated electrodes of micrometers in width, is discussed, as well as the static and in-line detection of sodium L-ascorbate. The analyte concentration is sensed through an electrocatalytic process in both static and in-line cases, by cyclic voltammetry and chronoamperometry, respectively. The findings presented in this work open novel opportunities for local surface modification of chip-integrated gold electrodes by redox-responsive polymers used, e.g., as electrochemical sensors.

This chapter is considered for publication in: *In-line Sensing of Sodium Ascorbate Using a Poly(ferrocenylsilane)-coated Microfluidic Device*, Kieviet, B.D., Schön, P.M., Vancso, G.J., **2016**, *Submitted*

4.1 Introduction

By integrating smart, stimulus-responsive polymers as functional elements, the performance capabilities of controlled fluid delivery and sensing in microfluidic devices can be greatly improved. Stimulus-responsive polymers^[1] are frequently used in microfluidics, both in poly(dimethylsiloxane)^[2-3] and in glass devices,^[4] as sensors^[5] and actuators.^[6-7] These polymers are often triggered by external stimuli, e.g., variations in temperature,^[8-11] solvent composition,^[12-13] pH^[14-15] or illumination with light.^[16] Of a different class are electroactive polymers as they enable device miniaturization and precise local addressability. Electroactive polymers^[17] have also been used in various microfluidic devices, for example, in electrically driven hydrogel actuators,^[18-19] in applying electro-responsive polymers for channel-width modulation^[20-21] or as diaphragm actuator.^[22] Of particular interest are redox-responsive polymers such as poly(ferrocenylsilane) (PFS) which offer fast, reversible switching and local addressability in combination with nanofabricated electrodes.^[23-29]

PFSs comprise a unique class of redox-active materials composed of alternating ferrocene and alkylsilane units in the main chain. They combine a high density of redox centers with excellent processability and reversible redox characteristics,^[30] in particular, it is of interest that the ferrocene moieties in the main chain render these polymers electroactive.^[31-34] Due to the substituent on the silane moiety in the main chain, PFS can be modified to have various properties such as water-solubility.^[35] Water soluble PFSs are used as polyelectrolyte coatings,^[36-38] or utilized as a redox-active gel for synthesis of silver^[39] or palladium nanoparticles.^[40] Actuators could be devised from PFS gels cross-linked poly(N-isopropylacrylamide), which reversibly collapse and expand upon isothermal oxidation.^[39]

Controlled in-chip electrode modification can be a challenge because of various limitations, such as minute volumes and channel confinement, leading to low diffusion and local accessibility of reagents. Mild procedures are required to prevent deterioration of the electrodes and channel walls. PFS can be grafted to surfaces by using sulfur-terminated PFS as self-assembled monolayers,^[41] by electrografting of PFS to gold^[42] or using a cysteamine monolayer for the covalent attachment of PFS chains to an electrode surface, employing amine alkylation reactions,^[43] encompassing a procedure which has been established recently in our group. Via this procedure, robust, dense, redox-active organometallic PFS films can be formed on gold substrates. These PFS polymer films can be employed as an electrochemical sensor, exhibiting high sensitivity, stability, and reproducibility.^[42-45]

In this chapter, we present a microfluidic device with gold-coated microchannels as electrodes, to which the redox-responsive PFS was covalently tethered. To test the sensing capabilities of the microfluidic device, vitamin C, in sodium L-ascorbate form,

was chosen as an analyte. Vitamin C is found in fruits and vegetables and is water-soluble. As nature's widely available antioxidant, it has been studied intensively.^[46-48] Therefore, ascorbic acid is an excellent test case for sensing applications,^[49] also where polymer-based sensors are concerned.^[50-52] Detection of ascorbic acid is mainly done in electrochemical systems,^[53] with detection limits up to 23 nM.^[54] However, these systems are mostly on macro-sized electrodes,^[55] while miniaturization, for example for lab on a chip applications, requires micro or nano-sized detectors, where detection limits are not even approaching the current macro-sized values,^[56] hence, new types of detection layers should be investigated. In this chapter, we fabricated a microfluidic device with integrated gold channel electrodes. Subsequently, PFS, with an iodopropyl sidechain, was utilized for inner-surface modification via alkylation of cysteamine-modified channel-electrodes. Finally, the use of suchlike modified microfluidic devices for electrochemical sensing was elaborated by the electrocatalytic determination of sodium ascorbate.

4.2 Device fabrication

4.2.1 Gold-coated microchannels

The fabrication and characterization of the glass microfluidic device with integrated gold channel electrodes is explained in more detail in this section. Glass wafers were cleaned with fuming nitric acid for 10 minutes and coated with a Cr/Au (10 nm/100 nm) etch mask using an evaporator. After spin-coating with photoresist, illumination with UV light for 8 s through a lithography mask and development in tetramethylammonium hydroxide solution for 60 s, the wafers were etched using a 25% HF solution for 20 minutes yielding 27 μm deep features. Afterwards, the resist was removed with acetone and the etch mask was removed with a gold etchant and a chromium etchant. Thereafter, the wafers were thoroughly rinsed with water and dried by spin-drying. The glass wafers, with microchannels, were cleaned again with fuming nitric acid for 10 minutes and spin-coated with a new layer of photoresist. Subsequent to illumination for 30 s through a second mask, and development, the wafers were immersed in buffered hydrofluoric acid solution (BHF) for 6 minutes to etch a 110 nm deep trench to embed the electrodes. 100 nm gold electrodes were evaporated on the photoresist. A 10 nm Cr layer was used as an adhesive layer for the glass. After a lift-off procedure in acetone using an ultrasonic bath, the wafers were cleaned using nitric acid for 10 minutes and Piranha solution (1:3 H_2O_2 : H_2SO_4 , 1 min) and subsequently bonded thermally (550°C, 1 h) to a cover glass wafer, on which access holes had been fabricated using a powderblast technique. The whole process is schematically shown in Figure 4.1. Both the images of the photomasks used and the step-by-step fabrication procedure can be found in the Appendix of this thesis.

Figure 4.1: Fabrication of the microfluidic device, from left to right: The glass wafer (a) was etched 27 μm deep with 25% HF (b), and 110 nm with BHF (c) using two different resist masks. After evaporation of the gold electrodes (100 nm) with Cr adhesive layer (10 nm) (d) the microfluidic device was thermally bonded to another glass wafer at 550°C (e), on which access holes were powderblasted (not shown).

Figure 4.2 shows a scanning electron microscopy (SEM) image of the gold-coated channel, as well as an atomic force microscopy (AFM) image of the electrode. Figure 4.2a displays an overview image of the section of interest of the device, and Figure 4.2b displays a close-up of the gold-covered channel. It can be seen that the electrode is successfully evaporated on top of the microfluidic channels, as is also shown by the AFM image in Figure 4.2c. The section plot, below the AFM image, corresponds to the blue line in the AFM image, and it can be seen that a 140 nm deep trench is etched by the BHF, therefore the electrode of height 110 nm is embedded into the glass, ensuring proper bonding with the top glass wafer in the fabrication step that followed.

Figure 4.2: SEM and AFM measurements of the fabricated microchannels coated with gold electrodes. a) Overview of the channel. b) Close-up of the edge of the electrode showing that the electrode is covering the channel (scale bars in both images is 10 μm). c) AFM measurement of the edge of the electrode (scale bar in nm), with corresponding cross-section. The gold is on the left side of the AFM image.

Prior to use with the polymer functionalization, the device performance was tested by analysis of potassium hexacyanoferrate(II), as shown in Figure 4.3. From this can be concluded that the device worked as electrochemical sensor by employing the gold-coated microchannels as working electrode.

Figure 4.3: Characterization of the electrochemical device using 2 mM $K_4Fe(CN)_6$ in 0.1 M $NaClO_4$ aqueous solution. The gold-coated microchannel was used as working electrode, a platinum wire was used as reference electrode and a second platinum wire as counter electrode.

4.2.2 Surface attachment of poly(ferrocenylsilane)

PFS, comprising iodopropyl and methyl sidegroups (PFS-I), was grafted to the cysteamine-modified gold surface of the microfluidic device, via amine alkylation. The grafting procedure is depicted in Scheme 4.1. The gold surface is first cleaned by oxygen plasma for 2 hours (1) and then covered with a monolayer of cysteamine (2) flowing a 10 mM solution (ethanol) overnight at $30 \mu L h^{-1}$. Afterwards, the PFS-I (5 mg mL^{-1} in tetrahydrofuran) is drop-casted and soaked into the channel by capillary forces, and, after drying with nitrogen flow, heated overnight at $50^\circ C$ under vacuum (3). The channels are subsequently rinsed thoroughly with tetrahydrofuran, a good solvent for PFS-I, to remove any physisorbed polymer. A more in-depth study of this process can be found in Ref. [43] for flat substrates and Ref. [57] for microchannels.

Scheme 4.1: Surface attachment of PFS. The gold surface is initially covered with a cysteamine monolayer (Ethanol, room temperature, overnight). Afterwards, the PFS is attached to the cysteamine-covered gold at $50^\circ C$ (THF, overnight).

To confirm the attachment of the PFS-I, cyclic voltammetry experiments were performed. Figure 4.4 shows two voltammograms, one of a non-coated device (red line) and one of a PFS-I-coated microfluidic device (blue line). The characteristic double wave of PFS can clearly be observed, proving that PFS is tethered to the working electrode. The double peak in the voltammogram is related to stepwise oxidation of the first (statistically every second) ferrocene in the main chain, followed by filling in the remaining sites at higher oxidation potentials.^[23]

Figure 4.4: Cyclic voltammograms of a PFS-I-coated (blue line) and non-coated (red line) microfluidic device. The double peak characteristic of PFS can clearly be observed. The gold-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode.

The faradaic current of the PFS layer clearly exceeds the capacitive current of the device. To test the kinetics of the PFS layer, the scan rate was also varied, the results are shown in Figure 4.2.

Figure 4.5: Scan-rate dependence of the second peak (I_p) of the PFS-I layer. The scan rate (v) was varied from 5 to 100 $mV s^{-1}$. The square-root dependence shows that the electrochemical response of the microfluidic device is diffusion controlled. The gold-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode.

Square-root dependence of the peak height on the scan rate is observed, hence can be concluded that the electrochemical response of the device is diffusion controlled. In contrast, on macro-sized electrodes, a linear relation was found in previous work from our group,^[42-43] which are characteristic for surface-confined layers.^[58] However, the ability of the electrolyte to diffuse into the polymer layer has a strong effect on the kinetic behavior of the film.^[59] Therefore, we attribute the diffusion control observed in our case to the confinement of the electrolyte due to the small dimensions compared to flat substrate/macro sized electrode studies. Adding to this effect is the large geometrical separation between the working electrode and counter and reference electrodes, namely around 18 mm, due to the design choices of the device.

4.3 Vitamin C sensing

4.3.1 Static sensing

The performance of the microfluidic device as electrochemical sensor was studied by electrochemical measurements in various concentrations of sodium ascorbate. To confirm the electrocatalytic behavior of the polymer layer, in stationary solution, cyclic voltammograms of the PFS-I-coated microfluidic device in the presence of sodium ascorbate were performed, as shown in Figure 4.6.

Figure 4.6: Cyclic voltammograms (Current I vs. potential E) of a PFS-I-coated microchannel, with increasing concentration of sodium ascorbate (0, 0.5, 1 and 2 mM, inner to outer lines). The electrocatalytic effect of the PFS is clear. The PFS-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode and no flow was applied.

The concentration of sodium ascorbate used was varied from 0.5 to 2 mM. A clear increase in peak current, when adding sodium ascorbate, can be observed, proving that the microfluidic device can detect the presence of sodium ascorbate. The electrocatalytic reaction proceeds as follows.^[60] After oxidation of the ferrocene moiety via the electrode potential, the ascorbate reduces ferric iron to ferrous iron, oxidizing into dehydroascorbic acid, allowing the ferrocene to once more oxidize to ferrocenium, resulting in an increase in current depending on the concentration of the ascorbate. However, due to the nature of the reaction, the amount of available ascorbate diminishes, resulting in lower current in each voltammetry cycle. To counteract this depletion a constant influx of ascorbate is required, leading to a steady state in current response. Hence, the electrochemical technique was changed from cyclic voltammetry to chronoamperometry and a constant ascorbate inflow was realized.

4.3.2 In-line vitamin C sensing

Figure 4.7 shows the chronoamperometric response of the PFS-modified microfluidic device, under application of various concentrations of sodium ascorbate over an order of magnitude from 0.5 mM to 5 mM, at +0.70 V vs. Ag/AgCl. The different amperometric responses are shifted in time to overlap, because, due to the microfluidic setup, the exact time of arrival of the sodium ascorbate at the PFS-covered microelectrode is not known beforehand. Furthermore, the contribution of the PFS layer itself is filtered from the signal. (See Figure 4.11 for the non-shifted curves).

Figure 4.7: Chronoamperometry curves of PFS-I (Current I vs. time t) with various concentrations of sodium ascorbate at +0.70 V vs. Ag/AgCl. The data is shifted in time to overlap where the solution entered the channel and in current to remove the trailing effect of the PFS-I oxidation. The concentrations used were 0, 0.5, 1, 2, 3, 4 and 5 mM (bottom to top), flow rate $5 \mu\text{L min}^{-1}$. Inset: calibration curve of the PFS sensor (Current I vs. concentration c), the slope is 3.59 nA mM^{-1} (R^2 0.98). The PFS-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode.

From Figure 4.7 several conclusions can be drawn. Firstly, from the shape of the curve it can be concluded that a second order diffusion process takes place, as the sodium ascorbate has to diffuse from the bulk fluid to the wall of the channel, since, due to the no-slip boundary condition, there is no flow at the wall. To reach a plateau value approximately 100 seconds are required. Secondly, the increase in plateau height increases linearly with concentration, shown in the inset in Figure 4.7, as can be expected from the Cottrell equation.^[58,61] The detection limit is in the $100 \mu\text{M}$ range, this compares with literature values of vitamin C detection in microfluidic devices: 2 mM in Ref. ^[62]; 0.5 mM in Ref. ^[63], $100 \mu\text{M}$ in Ref. ^[64] and $100 \mu\text{M}$ in Ref. ^[56]. To verify that the current response of the PFS is not influenced by the flow rate, cyclic voltammograms at various flow rates were performed, as well as amperometric sensing experiments, shown in Figure 4.8 and Figure 4.9, respectively.

Figure 4.8: Cyclic voltammograms of a PFS-I-coated microchannel, with increasing flow rate (0, 1, 2, 3, 4 and 5 $\mu\text{L min}^{-1}$). PFS-I showed no change in response when the flow rate was changed. The PFS-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode.

From these it can be concluded that the device is not influenced by the flow rate, apart from the time required to reach the plateau value. To further improve the sensitivity of the device, the availability of in-channel platinum counter and Ag/AgCl reference electrodes will be explored, to further miniaturize this microfluidic device.^[65-66] Furthermore, thickness control of the PFS in-chip layer will be investigated to improve the electron-transfer kinetics of the PFS layer. Moreover, other types of PFS will be investigated as active layers, such as a water soluble PFS.^[35,42]

Figure 4.9: Chronoamperometry of a PFS-I-coated microchannel with added sodium ascorbate (2 mM), and varying the flow rate (2 and 5 $\mu\text{L min}^{-1}$, blue and red line). It can be seen that the response is faster in the case of the higher flow rate. However, the plateau value is within the experimental error. The PFS-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode.

4.4 Conclusions

PFS was successfully tethered to cysteamine-modified gold-coated microchannel electrodes. The microfluidic device was utilized to sense sodium ascorbate, with a detection limit of 0.5 mM concentration. This indicates that PFS is a strong candidate as active layer for electrochemical sensors in microfluidic devices. In future work, by controlling the layer thickness and device dimensions, we anticipate higher sensitivity of the device.

4.5 Experimental Section

Reagents and solvents: MEMPax Glass wafers (100 mm diameter, 0.5 mm thickness) were purchased from Schott. From Arch Chemicals Inc., Olin OIR 907-17 was used as photoresist, tetramethylammonium hydroxide solution as developer. 25% HF solution was made by diluting 50% HF solution purchased from BASF. Chromium etchant was purchased from Technic. BHF (1:7 HF:NH₄F) was purchased from Honeywell. Nitric acid was purchased from KMG Chemicals. Acetone, sulfuric acid and hydrogen peroxide were purchased from BASF. Potassium iodide, iodine, potassium ferrocyanide, cysteamine, sodium perchlorate and sodium ascorbate were purchased from Sigma-Aldrich. Tetrahydrofuran was purchased from Biosolve. Ethanol was purchased from Merck. All chemicals were analytical grade and used as received, except where noted otherwise. The synthesis of PFS-I is described elsewhere.^[37,67]

Device fabrication and characterization: Chromium and gold were evaporated at 0.05 nm s⁻¹ and 0.2 nm s⁻¹, respectively, using a Balzers BAK 600. UV exposure was performed with an EVG 620 at 12 mW cm⁻². SEM experiments were performed using a JEOL JSM-6330F SEM, AFM experiments were done with a Bruker Dimension 3100 equipped with a hybrid scanner and a NanoScope V1a controller, using 'tapping mode' and a silicon nitride tip.

Figure 4.10: Electrochemical setup of the microfluidic device. 1) Needle contacting the gold pads leading to the gold-coated microchannel. 2) Inlet. 3) Ag/AgCl reference electrode. 4) Platinum counter electrode. Vitamin C pill is shown for size (21.8 mm in length).

Electrochemical experiments: Cyclic voltammetry and chronoamperometry were performed using a CH instruments 852D potentiostat. “In-chip” measurements were done as follows. The chip was first filled with the electrolyte solution (0.1 M NaClO₄ in H₂O) using a syringe pump, this is the electrolyte used in all experiments. A platinum wire and a Ag/AgCl electrode (BASi) were gently immersed into the now-formed droplets at the two outlets and used as counter and reference electrode, respectively. Finally, using a needle, the gold-coated channel was contacted via microfabricated contact pads and used as working electrode. The whole setup was placed on a stabilizing table. A photograph of the microfluidic device in the set-up can be found in Figure 4.10.

4.6 References

- [1] M. A. Cohen Stuart, W. T. S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov, S. Minko, *Nature materials* **2010**, 9, 101-13 | 10.1038/nmat2614
- [2] J. Zhou, A. V. Ellis, N. H. Voelcker, *Electrophoresis* **2010**, 31, 2-16 | 10.1002/elps.200900475
- [3] I. Wong, C.-M. Ho, *Microfluid. Nanofluid.* **2009**, 7, 291-306 | 10.1007/s10404-009-0443-4
- [4] B. D. Kieviet, P. M. Schön, G. J. Vancso, *Lab on a chip* **2014**, 14, 4159-4170 | 10.1039/c4lc00784k
- [5] Nuriman, B. Kuswandi, W. Verboom, *Sensors Actuators B: Chem.* **2011**, 157, 438-443 | 10.1016/j.snb.2011.04.084
- [6] H. Arora, R. Malik, L. Yeghiazarian, C. Cohen, U. Wiesner, *J. Polym. Sci., Part A: Polym. Chem.* **2009**, 47, 5027-5033 | 10.1002/pola.23555
- [7] G. Londe, A. Chunder, A. Wesser, L. Zhai, H. Cho, *Sensors Actuators B: Chem.* **2008**, 132, 431-438 | 10.1016/j.snb.2007.10.052
- [8] A. Richter, D. Kuckling, S. Howitz, T. Gehring, K. Arndt, *Journal of Microelectromechanical Systems* **2003**, 12, 748-753 | 10.1109/JMEMS.2003.817898
- [9] C. Yu, S. Mutlu, P. Selvaganapathy, C. H. Mastrangelo, F. Svec, J. M. J. Fréchet, *Anal. Chem.* **2003**, 75 (8), 1958-1961 | 10.1021/ac026455j
- [10] T. Saitoh, N. Suzuki, T. Furuse, M. Hiraide, *Talanta* **2009**, 80, 1012-5 | 10.1016/j.talanta.2009.07.001
- [11] N. Idota, a. Kikuchi, J. Kobayashi, K. Sakai, T. Okano, *Adv. Mater.* **2005**, 17, 2723-2727 | 10.1002/adma.200402068
- [12] H. Li, F. Lai, R. Luo, *Langmuir* **2009**, 25, 13142-50 | 10.1021/la901833m
- [13] M. F. Mora, C. E. Giacomelli, C. D. Garcia, *Anal. Chem.* **2007**, 79, 6675-81 | 10.1021/ac070953g
- [14] D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss, B.-h. Jo, *Nature* **2000**, 404, 588-590 | 10.1038/35007047
- [15] M. Salim, P. C. Wright, S. L. McArthur, *Electrophoresis* **2009**, 30, 1877-87 | 10.1002/elps.200800619
- [16] Z. Walsh, S. Scarmagnani, F. Benito-López, S. Abele, F.-Q. Nie, C. Slater, R. Byrne, D. Diamond, B. Paull, M. Macka, *Sensors Actuators B: Chem.* **2010**, 148, 569-576 | 10.1016/j.snb.2010.04.048
- [17] R. Shankar, T. K. Ghosh, R. J. Spontak, *Soft Matter* **2007**, 3, 1116 | 10.1039/b705737g

- [18] G. H. Kwon, Y. Y. Choi, J. Y. Park, D. H. Woo, K. B. Lee, J. H. Kim, S.-H. Lee, *Lab on a chip* **2010**, *10*, 1604-10 | 10.1039/b926443d
- [19] G. H. Kwon, G. S. Jeong, J. Y. Park, J. H. Moon, S.-H. Lee, *Lab on a chip* **2011**, *11*, 2910-5 | 10.1039/c1lc20288j
- [20] A. K. Price, K. M. Anderson, C. T. Culbertson, *Lab on a chip* **2009**, *9*, 2076-84 | 10.1039/b823465e
- [21] C. Murray, D. McCoul, E. Sollier, T. Ruggiero, X. Niu, Q. Pei, D. D. Carlo, *Microfluid. Nanofluid.* **2012**, *14*, 345-358 | 10.1007/s10404-012-1055-y
- [22] F. Xia, S. Tadigadapa, Q. M. Zhang, *Sensors and Actuators A: Physical* **2006**, *125*, 346-352 | 10.1016/j.sna.2005.06.026
- [23] R. Rulkens, A. J. Lough, I. Manners, S. R. Lovelace, C. Grant, W. E. Geiger, *J. Am. Chem. Soc.* **1996**, *118*, 12683-12695 | 10.1021/ja962470s
- [24] G. R. Whittell, M. D. Hager, U. S. Schubert, I. Manners, *Nature materials* **2011**, *10*, 176-88 | 10.1038/nmat2966
- [25] R. A. Musgrave, A. D. Russell, I. Manners, *Organometallics* **2013**, *32*, 5654-5667 | 10.1021/om400488k
- [26] J. Duvigneau, E. Kutnyanszky, I. Y. Phang, H.-J. Chung, H. Wu, L. Dos Ramos, T. Gädt, S. F. M. Yusoff, M. A. Hempenius, I. Manners, G. J. Vancso, *Polymer* **2014**, *55*, 2716-2724 | 10.1016/j.polymer.2014.04.021
- [27] M. Erhard, K. Lam, M. Haddow, G. R. Whittell, W. E. Geiger, I. Manners, *Polymer Chemistry* **2014**, *5*, 1264 | 10.1039/c3py01232h
- [28] A. D. Russell, R. A. Musgrave, L. K. Stoll, P. Choi, H. Qiu, I. Manners, *J. Organomet. Chem.* **2015**, *784*, 24-30 | 10.1016/j.jorganchem.2014.10.038
- [29] G. Cambridge, M. J. Gonzalez-Alvarez, G. Guerin, I. Manners, M. A. Winnik, *Macromolecules* **2015**, *48*, 707-716 | 10.1021/ma502279b
- [30] I. Manners, *Chem. Commun.* **1999**, 857-865 | 10.1039/a810043h
- [31] X. Feng, B. D. Kieviet, J. Song, P. M. Schön, G. J. Vancso, *Appl. Surf. Sci.* **2014**, *292*, 107-110 | 10.1016/j.apsusc.2013.11.087
- [32] M. Péter, M. A. Hempenius, E. S. Kooij, T. A. Jenkins, S. J. Roser, W. Knoll, G. J. Vancso, *Langmuir* **2004**, *20*, 891-897 | 10.1021/la035656v
- [33] X. Sui, X. Feng, M. A. Hempenius, G. J. Vancso, *J. Mater. Chem. B* **2013**, *1*, 1658 | 10.1039/C3TB00209H
- [34] I. Manners, *J. Inorg. Organomet. Polym.* **1993**, *3* (3), 185-196 | 10.1007/BF00683928
- [35] M. A. Hempenius, M. Péter, N. S. Robins, E. S. Kooij, G. J. Vancso, *Langmuir* **2002**, *18*, 7629-7634 | 10.1021/la011811z
- [36] M. A. Hempenius, F. F. Brito, G. J. Vancso, *Macromolecules* **2003**, *36*, 6683-6688 | 10.1021/ma034432g
- [37] M. A. Hempenius, N. S. Robins, R. G. H. Lammertink, G. J. Vancso, *Macromol. Rapid Commun.* **2001**, *22*, 30-33 | 10.1002/1521-3927(20010101)22:1<30::AID-MARC30>3.0.CO;2-J
- [38] Y. Ma, W.-F. Dong, E. S. Kooij, M. A. Hempenius, H. Möhwald, G. J. Vancso, *Soft Matter* **2007**, *3*, 889 | 10.1039/b702132a
- [39] X. Sui, X. Feng, A. Di Luca, C. A. van Blitterswijk, L. Moroni, M. A. Hempenius, G. J. Vancso, *Polymer Chemistry* **2013**, *4*, 337 | 10.1039/c2py20431b
- [40] B. Zoetebier, M. A. Hempenius, G. J. Vancso, *Chem. Commun.* **2014**, 1-4 | 10.1039/C4CC06988A
- [41] M. Peter, R. G. H. Lammertink, M. A. Hempenius, M. van Os, M. W. J. Beulen, D. N. Reinhoudt, W. Knoll, G. J. Vancso, *Chem. Commun.* **1999**, 359-360 | 10.1039/a809259a

- [42] X. Feng, X. Sui, M. A. Hempenius, G. J. Vancso, *J. Am. Chem. Soc.* **2014**, *136*, 7865-7868 | 10.1021/ja503807r
- [43] X. Sui, X. Feng, J. Song, M. a. Hempenius, G. J. Vancso, *J. Mater. Chem.* **2012**, *22*, 11261 | 10.1039/c2jm30599b
- [44] J. Song, G. J. Vancso, *Langmuir* **2011**, *27*, 6822-9 | 10.1021/la200494r
- [45] X. Feng, A. Cumurcu, X. Sui, J. Song, M. A. Hempenius, G. J. Vancso, *Langmuir* **2013**, *29*, 7257-65 | 10.1021/la304498g
- [46] C. Rice-Evans, N. Miller, G. Paganga, *Trends Plant Sci.* **1997**, *2*, 152-159 | 10.1016/S1360-1385(97)01018-2
- [47] I. D. Podmore, H. R. Griffiths, K. E. Herbert, N. Mistry, P. Mistry, J. Lunec, *Nature* **1998**, *392*, 559 | 10.1097/00006454-199809000-00030
- [48] M. Valko, C. J. Rhodes, J. Moncol, M. Izakovic, M. Mazur, *Chem. Biol. Interact.* **2006**, *160*, 1-40 | 10.1016/j.cbi.2005.12.009
- [49] D. W. Kimmel, G. LeBlanc, M. E. Meschievitz, D. E. Cliffler, *Anal. Chem.* **2012**, *84* (2), 685-707 | 10.1021/ac202878q
- [50] A. Bossi, S. A. Piletsky, E. V. Piletska, P. G. Righetti, A. P. Turner, *Anal. Chem.* **2000**, *72*, 4296-300 | 10.1021/ac000185s
- [51] C.-J. Weng, Y.-S. Jhuo, Y.-L. Chen, C.-F. Feng, C.-H. Chang, S.-W. Chen, J.-M. Yeh, Y. Wei, *J. Mater. Chem.* **2011**, *21*, 15666 | 10.1039/c1jm12422f
- [52] N. Malashikhina, V. Pavlov, *Biosens. Bioelectron.* **2012**, *33*, 241-246 | 10.1016/j.bios.2012.01.011
- [53] A. M. Pisoschi, A. Pop, A. I. Serban, C. Fafaneata, *Electrochim. Acta* **2014**, *121*, 443-460 | 10.1016/j.electacta.2013.12.127
- [54] G. M. Greenway, P. Ongomo, *Analyst* **1990**, *115*, 1297-1299 | 10.1039/AN9901501297
- [55] B. K. Chethana, Y. Arthoba Naik, *Analytical Methods* **2012**, *4*, 3754 | 10.1039/c2ay25528f
- [56] R. F. Carvalhal, M. Sima, M. Helena, D. O. Piazzetta, A. L. Gobbi, L. T. Kubota, *Anal. Chem.* **2010**, *82*, 1162-1165 | 10.1021/ac902647r
- [57] L. Dos Ramos, G. Lajoinie, B. D. Kieviet, S. de Beer, M. Versluis, M. A. Hempenius, G. J. Vancso, **2015**, *submitted*.
- [58] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*. John Wiley & Sons, Inc.: **2001**; p 864.
- [59] X. Wang, L. Wang, J. Wang, T. Chen, *The Journal of Physical Chemistry B* **2004**, *108*, 5627-5633 | 10.1021/jp0369903
- [60] J. Du, J. J. Cullen, G. R. Buettner, *Biochim. Biophys. Acta* **2012**, *1826*, 443-457 | 10.1016/j.bbcan.2012.06.003
- [61] $i(t) = n F A D_0^{1/2} C_0 \pi^{-1/2} t^{1/2}$.
- [62] J. Wang, M. P. Chatrathi, B. Tian, R. Polsky, *Anal. Chem.* **2000**, *72*, 2514-2518 | 10.1021/ac991489l
- [63] F. Xia, W. Jin, X. Yin, Z. Fang, *J. Chromatogr. A* **2005**, *1063*, 227-233 | 10.1016/j.chroma.2004.12.022
- [64] J. Qiu, P. Hu, R. Liang, *Anal. Sci.* **2007**, *23*, 1409-1414 | 10.2116/analsci.23.1409
- [65] E. V. Dydek, M. V. Petersen, D. G. Nocera, K. F. Jensen, *Lab on a chip* **2012**, *12*, 1431 | 10.1039/c2lc21195e
- [66] E. V. Dydek, M. V. Petersen, D. G. Nocera, K. F. Jensen, *J. Electrochem. Soc.* **2012**, *159*, H853-H856 | 10.1149/2.007211jes
- [67] M. A. Hempenius, G. J. Vancso, *Macromolecules* **2002**, *35*, 2445-2447 | 10.1021/ma0119629

Appendix

Figure 4.11: Non-shifted chronoamperometry curves of PFS-I with various concentrations of sodium ascorbate. The concentrations used were 0, 0.5, 1, 2, 3, 4 and 5 mM (bottom to top, from the right). The PFS-coated microchannel was used as working electrode, Ag/AgCl was used as reference electrode, platinum wire as counter electrode.

Geometry-dependent insertion-forces on particles in swollen polymer brushes

We present molecular dynamics simulations in which we determine the forces on cubes, rods, discs and spheres that are included in polymer brushes at different distances from the anchoring surface. We show that one can predict the forces on such particles by combining multiple theoretical models that consider (1) the excluded volume interaction caused by the presence of a polymer-cavity in the brush, (2) the surface tension due to the created particle-polymer interface, and (3) a correction to the pressure gradient over the particle-surface induced by a streamlining effect on the polymer paths. Our description of the distance-dependent inclusion free energy and force for spherical and non-spherical particles in polymer brushes might ultimately aid in the development of brush-based sensors and filters, which rely on enhancing or preventing the entrapment of arbitrarily shaped particles.

This chapter has been published in: S. de Beer, L.I.S. Mensink, B.D. Kieviet, *Macromolecules* **2016**, *49* (3), 1070–1078 | [10.1021/acs.macromol.5b01960](https://doi.org/10.1021/acs.macromol.5b01960). In this work, the molecular dynamics simulations were set up by LM, auxiliary simulations, data analysis and models were done by BDK. SdB supervised the project.

5.1 Introduction

Mucus consists of proteins decorated with saccharide chains immersed in an aqueous liquid.^[1] It forms a soft, dilute polymeric barrier in, e.g., the respiratory or intestinal system. This polymeric barrier acts as a filter allowing nutrient-uptake, while hindering the absorbance of bacteria and toxic materials.^[2-3] Being able to predict particle-inclusion in these barrier-like solvated polymeric systems might ultimately aid in the design of efficient medication or help predict potential nano-toxicity of substances. In addition, the study of the properties of particle-polymer systems is relevant for a myriad of other applications,^[4] such as micro-reactors,^[5] filters,^[6-8] scaffolds,^[9] (nano-)sensors,^[10-11] “pick-up and place” systems,^[12-13] lubricants,^[14] antifouling^[15-17] and antibacterial^[18] surfaces, and (smart) microfluidic systems.^[19-20] For many of these applications, the particles are trapped or immobilized using so-called polymer brushes.^[5,9-12,14,20] Also, the polymers in mucus, that are close to the interface with another medium, can stretch out and form a brush-like configuration.^[21] Therefore, the static and dynamic behavior of particles in and on polymer brushes have been studied extensively in theory,^[16,22-29] simulations^[30-37] and experiments.^[28,38-42]

Polymer brushes are formed when polymers are constrained at one end to an interface at a high density.^[43-45] When the distance between anchor points in the brush is smaller than the radius of gyration of the free polymer, the polymers will stretch away from the anchoring plane due to steric interactions with the neighboring polymers. The degree of stretching depends on the presence and quality of the solvent. Polymer brushes form a dense film on the anchoring surface when they are dry or in a poor solvent, while the polymer chains stretch stronger under good solvent conditions, because the solvent is absorbed in the brush. Brushes are not only helpful in controlling particle absorption and immobilization, but can also act as efficient lubricants^[46-47] or aid in oil recovery.^[48] The introduction of particles in a polymer brush will change the free energy of the system^[22] and the particles will be expelled from or included in the brush depending on their size,^[22] and the solvent-mediated^[27] polymer-particle interactions,^[49] which can be of enthalpic and entropic origin.

Molecular dynamics (MD) and Monte Carlo (MC) simulations have been particularly helpful in understanding and predicting particle inclusion in polymer brushes, because in these simulations it is straightforward to track and visualize particles while monitoring the forces on them. Yaneva et al.^[31] found that particles below a threshold size of $b^* \sim \sigma_g^{-2/3}$ (with σ_g being the grafting density) will move freely and distribute in the brush. For sizes between b^* and a $b_{\max}$, the particles reside in the dilute top-part of the brush, while particles of sizes larger than this $b_{\max}$ do not enter the brush at all. This observation is in qualitative agreement with theoretical predictions based on the change in free energy of mixing for including nanoparticles in a brush^[23,49] taking into account the parabolic density profile of a brush, as first proposed by Semenov:^[50-52] For particles smaller than b^* , the

particles distribute themselves over the entire brush, because the entropy gain of mixing is larger than the energy penalty due to the changes in the polymer configuration induced by the inclusion. This energy penalty depends on the local polymer-density in the brush, such that larger particles ($b^* < b < b_{\max}$) will only move into the low-density top-layer up to a penetration depth δ , while very large particles of size $b > b_{\max}$ will remain outside the brush. To quantify the energy penalty of including a particle in a brush, Milchev et al.^[30] and Merlitz et al.^[33] performed extensive simulations of spherical particles of different radii r in brushes of different grafting densities. They found a quadratic dependence of the inclusion free energy on the particle-radius ($E_{\text{incl}} \sim r^2$) when the polymer density is low, while for high densities they observed that $E_{\text{incl}} \sim r^3$. Their results imply that the presence of an excluded volume (caused by the particle) dominates E_{incl} at high densities, and that at low densities E_{incl} is determined by the surface tension caused by the introduced particle-polymer interface.

So far, most simulations focused on spherical particles in brushes.^[30-36] However, realistic particles are almost never perfect spheres. For example, the fibers of glass wool, carbon nano-tubes and proteins tend to have more rodlike shapes, whereas most salt crystals are cubic. Since the surface-to-volume ratio of non-spherical objects is different from simple spheres, we anticipate that E_{incl} will also change. Moreover, for rotationally asymmetric particles, the relative orientation in the brush will also affect E_{incl} .^[23] In fact, it was recently shown that the preferred orientation of rodlike particles can be horizontal or vertical depending on the grafting density.^[53] Therefore and in view of potential applications, it is important to characterize how the particle's geometry and orientation can affect the inclusion free energy upon introduction of the particles in brushes.

In this chapter, we present MD simulations of polymer brushes that are penetrated by differently shaped particles such as cubes, rods, discs and spheres at various orientations with respect to the polymer-anchoring plane. We monitor the forces on the particles at different indentation-depths and compare them to a theoretical description for particles in polymer solutions, based on a model description proposed by Louis et al.^[54] This description links the inclusion free energy to the osmotic pressure and the effective adsorption of polymers at the particle surface via an excluded volume interaction and a surface tension contribution to the total energy. We slightly alter this description by introducing a correction factor as proposed by Kim and O'Shaughnessy^[23] which incorporates the effect of the streamlined polymer paths specifically for polymers in brushes. Both the osmotic pressure and the effective adsorption depend on the local monomer density,^[54] which we extract from our simulations. We determine the exact relation between pressure, adsorption and the local monomer density by two methods. One method is based on scaling relations proposed by de Gennes^[55] for semi-dilute polymers (SC method) and the other method is a bottom-up approach in which we directly determine the relations via simulations of free polymers in solution (Bulk method). Both

methods qualitatively capture the force vs distance curves as we observe them in our simulations. However, the SC method outperforms the Bulk method. We attribute the better performance of the SC method to the possibility of fine-tuning the agreement via the fitting parameters in the prefactors. The Bulk method overestimates the inclusion force by 20-30% which we ascribe to an overestimation of the excluded volume due to our model-assumption of the particles being hard spheres.

5.2 Model and Methods

5.2.1 Molecular Dynamics simulations

In our MD simulations, we employ a generic coarse-grained model as first described by Kremer and Grest.^[56] The Kremer-Grest model has been successful in qualitatively reproducing the generic physical behavior of bulk,^[57] surface-adsorbed^[58] and end-grafted polymers.^[59] The pairwise, non-bonded interactions are described using the Lennard-Jones (LJ) potential, V_{LJ} :

$$V_{LJ} = 4\epsilon \left(\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right) \quad (r < r_{\text{cut}}), \quad (5.1)$$

in which r_{ij} is the distance between two interacting particles, ϵ represents the energy well depth, and σ is the distance at which the potential V_{LJ} is zero. The reduced Lennard-Jones units can be translated to real values for polymers such as poly(ethylene) by setting ϵ to 30 meV and σ to 0.5 nm.^[57]

We describe the bonded interaction between consecutive polymer beads through elastic springs by a Finitely Extensible Nonlinear Elastic (FENE) potential in combination with the LJ potential resulting in:

$$V_{KG} = -0.5kR_0^2 \ln \left(1 - \left(\frac{r_{ij}}{R_0} \right)^2 \right) + 4\epsilon \left(\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right) + \epsilon. \quad (5.2)$$

Our LJ interaction parameters are $\epsilon = 1$ and $\sigma = 1$ and we use a stiffness of $k = 30 \epsilon \sigma^{-2}$ and a maximum extension $R_0 = 1.5 \sigma$, which ensures that there is no bond-crossing.^[56] A polymer bead represents typically 3-4 monomers. Therefore, the unit of mass m is 10^{-22} kg and thus the unit of velocity v represents 7 m s^{-1} .^[57]

Figure 5.1 shows an example of our simulation cell. We model a polymer brush by end-grafting 168 chains ($N = 100$ beads) onto a surface ($32.40 \sigma \times 33.26 \sigma$) at a grafting density σ_g of 0.16 chains σ^{-2} . Since $R_g = 6.72 \sigma$ for free polymers of $N = 100$, our grafting density is approximately 22x the critical grafting density σ_g^* for brush formation $\sigma_g^* = \pi^{-1} R_g^{-2}$ and, thus, we are beyond the mushroom-to-brush transition and deep into the brush regime.^[60] A grafting density of 22x σ_g^* is well within the experimental range.^[61] The solvent is modeled implicitly via purely repulsive interactions between non-consecutive beads such that we approximate good (athermal) solvent conditions for which the interaction between polymers is also effectively repulsive. This is achieved by setting the

cutoff-radius r_{cut} at $2^{1/6} \sigma$. The substrate consists of a single layer of atoms on a hexagonal lattice with a lattice constant of 1.2σ . The wall atoms are connected to their lattice sites through harmonic springs ($k_{\text{lat}} = 16 \epsilon \sigma^{-2}$) and to their neighbors using an anharmonic spring ($k_{\text{ww}} = 30 \epsilon \sigma^{-2}$), ensuring a maximum displacement of the wall atoms by 0.4σ . The interaction between the polymer beads and the wall atoms is purely repulsive, to prevent local changes in the conformational state of the brush^[62] close to the wall due to attractive interactions.

Figure 5.1: Graphical representation of a diagonally placed rod in a polymers brush. Black particles represent the rod, grey spheres form the substrate and the brush is presented in orange. The image is rendered using visual molecular dynamics (VMD).^[63]

A Langevin thermostat is employed to keep the temperature constant at $1.5 \epsilon k_B^{-1}$ using a damping coefficient of $1.0 \tau^{-1}$. The equations of motion are solved using the velocity Verlet algorithm, as it is implemented in LAMMPS,^[64] using a timestep of 0.005τ . Simulations are performed in the NVT ensemble. The nanoparticles are kept statically in the brushes at different distances from the grafting surface. We define the distance D to be the distance between the wall atoms and the position of the centre of mass of the nanoparticle. The nanoparticles are moved to the D of our choice, at a constant velocity v of $0.005 \sigma \tau^{-1}$ in the direction towards or from the surface. The particles are then kept in place by not updating the nanoparticle's positions in response to applied external forces. The nanoparticles interact repulsively with both the substrate and the polymers ($r_{\text{cut}} = 2^{1/6} \sigma$). After a relaxation time of 10^6 timesteps (5000τ), which we confirmed to be long enough to ensure equilibrium, the average force on the particle at the fixed distance is monitored over 10^6 timesteps (5000τ). We tested that our results are not affected by the finite box size by indenting the particle in a brush on a surface of size 62.4σ by 70.7σ as well (same grafting density). Within 4%, which is within the statistical noise, the forces in this larger system are the same as the forces in our default system.

The simulated nanoparticles consist of a group of n fixed, individual particles, all with a mass of 1 m. The shapes and orientations are given in Table 5.1 as well as n , the surface area S , volume V and the correction factor ξ_v (including the standard error SE of the mean with a 95% confidence interval, see next section). The spheres are made up of randomly positioned particles, to prevent faceting-effects on the inclusion force. The cube has a simple cubic structure. The plate and rod are built in a hexagonal lattice.

Table 5.1: Features of the nanoparticles

	Shape	Size (σ)	Orientation	n	S (σ^2)	V (σ^3)	$\xi_v \pm SE$
Sphere	5	n/a	65	137.30	106.33	1.18 ± 0.00
		8	n/a	268	333.11	337.54	1.21 ± 0.01
Cube	6	Face down Point down	216	269.61	305.38	1.08 ± 0.02 1.12 ± 0.02
		8	Face down Point down	512	463.89	666.80	1.05 ± 0.03 1.05 ± 0.03
Disc	7x2	Horizontal Vertical	74	144.20	115.20	1.23 ± 0.01 1.12 ± 0.02
		9x2	Horizontal Vertical	122	207.84	180.78	1.27 ± 0.02 1.11 ± 0.01
Rod	3x18	Horizontal	126	254.13	201.70	1.27 ± 0.04
			Diagonal Vertical				1.15 ± 0.01 1.00 ± 0.01

5.2.2 Theoretical model

To predict the force F on a particle that is kept in the polymer brush, we compute the derivative of the free energy E_{incl} required to include the particle in the brush with respect to the distance from the anchoring-surface D .^[65] $F = -dE_{\text{incl}}/dD$. Since our particles are not allowed to translate or rotate, no additional correction to this equation are needed.^[66] As shown by Louis et al.^[54], the inclusion free energy of particles in polymeric systems can be described by:

$$E_{\text{incl}} = \Pi \cdot V + \gamma \cdot S, \quad (5.3)$$

with Π being the osmotic pressure, V the volume of the particle, γ the surface tension, and S the surface area of the particle. The first term represents the work to create a cavity of volume V in the polymeric system and the second term describes the surface energy of the created polymer-particle interface. The surface tension depends on the osmotic pressure and can be computed via:^[54]

$$\gamma = -\Pi \cdot \hat{\Gamma} + \int_0^{\rho} \Pi(\rho') \left(\frac{\partial \hat{\Gamma}}{\partial \rho'} \right) d\rho', \quad (5.4)$$

where $\hat{\Gamma}$ is the 'relative adsorption'^[54] and captures the effect of the change in density due to the creation of the interface and can be determined by:^[67]

$$\hat{f} = \int_0^\infty h(z) dz, \quad (5.5)$$

$$\text{with } h(z) = \frac{\rho(z)}{\rho} - 1 \quad (5.6)$$

in which ρ_z is the monomer density profile at distance z from the particle surface, and ρ the monomer density in the bulk.

Kim and O'Shaughnessy^[23] proposed that, for the specific case of polymer brushes with particles that are larger than the blob size, the inclusion free energy needs to be adjusted. Large particles force the trajectories of the polymer chains to deviate (on average) from the stretching direction, which induces a deviation of the density gradient over the particle-surface from the density gradient it would have if the brush-monomers would locally behave as monomers of polymers in bulk solution. The authors of Ref. ^[23] show that this deviation results in a correction factor ξ_v for the inclusion free energy, such that Equation 5.3 becomes

$$E_{\text{incl}} = \xi_v \Pi \cdot V, \quad (5.7)$$

for inclusions deep in the brush, where the excluded volume interactions dominate. The correction factor ξ_v should be of order unity^[23] and depends on the shape and orientation of the nanoparticle. We determine ξ_v directly from our simulations. To do so, we extract E_{incl} for deep inclusion of the different shapes and orientations by integration of the inclusion force F from the inclusion distance D to infinity. The correction factor is the deviation from the linear relation between E_{incl} and V with slope Π (see Results and Discussion section). We determine the slope (Π) from the E_{incl} for the vertically positioned rod, for which the deflections in the polymer path are the smallest such that the correction factor can be assumed to be 1.^[23] Note that in simulations of free particles, the vertical cylinder was found to be the optimum shape for (close to) purely repulsive interactions and low concentrations.^[36,68] For attractive interactions and / or higher concentrations the vertical cylinder is not the optimum shape and, thus, under these conditions, one should allow for a correction factor ξ_v lower than 1. We note that the surface energy term in Equation 5.3 might need a similar correction factor. However, we found that these corrections are smaller than the stochastic noise in our simulations.

The volume V and surface area S of the particles are determined from the positions of the individual particles in the simulated nanoparticle. Since the inclusion free energy arises due to the effect of the excluded volume for the polymer beads, we define the surface of the nanoparticle to represent the boundary of the region in which the center of mass of polymer beads cannot come. Therefore, each point particle is assumed to be a sphere of fixed volume with radius σ .^[31,33] The total excluded volume and surface area are calculated by merging the volumes of the individual particles using SolidWorks (2014, SP4, Dassault Systèmes, HQ: Waltham, Massachusetts, USA).

Both Π and $\hat{\Gamma}$ (and thus γ) depend on the local monomer density ρ in the brush, which changes with the distance from the anchoring-surface D . We can extract $\rho(D)$ from our simulations. Nevertheless, to compare the forces on the simulated particles in the brushes to the theoretical force described above, we need to find the approximate relation between the ρ and both Π and $\hat{\Gamma}$. To do so, we employ two methods, which will be explained in more detail in the next sections. In the first method (SC method), the density dependence of Π and $\hat{\Gamma}$ is approximated using scaling arguments of de Gennes.^[55] In the second method (Bulk method), we employ a bottom-up approach and determine the density dependence of Π and $\hat{\Gamma}$ from bulk polymer simulations.

Because the density varies nonlinearly over the height of the particles, we compute an effective density for each particle and orientation at each D . This was done as follows: for each slice of $\Delta h = 0.25 \sigma$ over the height h of the particle in z , the local (outer) surface of the slice, ΔS , and volume of the slice, ΔV , are calculated. Subsequently, the density at this slice is scaled, in a first-order approximation, by $\Delta S/S$ and $\Delta V/V$, resulting upon integration in two effective densities for the surface and volume term, respectively. Naturally, $\rho_{\text{eff}}(D)$ is different for volume-weighting compared to surface-weighting, such that at each D and for each particle and orientation there are two effective densities: one for volume effects (first term in Equation 5.3) and one for surface effects (second term Equation 5.3). These two $\rho_{\text{eff}}(D)$'s are both used as input for the two methods described below.

SC Method

Within the scaling arguments of de Gennes,^[55] the osmotic pressure is related to the monomer density of free polymers in solution by:^[69]

$$\Pi^{\text{SC}} = \alpha \left(\frac{k_b T}{a_m^3} \right) \rho^{\frac{3\nu}{3\nu-1}} = \alpha \left(\frac{k_b T}{a_m^3} \right) \rho^{2.31}, \quad (5.8)$$

with ν the Flory exponent of $\nu = 0.588$, a_m the effective monomer size, and α a fitting parameter. This scaling relation has been shown to agree better with experimental observations^[69] than the mean field approximation^[55] of $\Pi^{\text{MF}} \sim \rho^2$. To express $\hat{\Gamma}^{\text{SC}}$ as a function of the local monomer density, we employ that $-\hat{\Gamma}^{\text{SC}}$ is directly related to the correlation length $\xi(\rho)$ ^[54] or blob size,^[55] which depends on the density via $\xi(\rho) \sim \rho^{-\nu/(3\nu-1)} \sim \rho^{-0.77}$, such that $\hat{\Gamma}^{\text{SC}} = \beta \rho^{-0.77}$, with β being a fitting parameter. Substituting this equation and Equation 5.8 into Equation 5.4 results in the surface tension being described by:

$$\gamma^{\text{SC}} = -\frac{3}{2} \Pi^{\text{SC}} \hat{\Gamma}^{\text{SC}} = -\alpha \left(\frac{k_b T}{a_m^3} \right) \beta \rho^{1.54}. \quad (5.9)$$

To obtain the final expression for $E_{\text{incl}}^{\text{SC}}$ the equations for Π^{SC} (Equation 5.8) and γ^{SC} (Equation 5.9) can be substituted in Equation 5.3 to obtain:

$$E_{\text{incl}}^{\text{SC}} = \xi_v \alpha \left(\frac{k_b T}{a_m^3} \right) \rho^{2.31} V - \alpha \left(\frac{k_b T}{a_m^3} \right) \beta \rho^{1.54} S. \quad (5.10)$$

Bulk Method

In a recent publication, it was shown that the exponent of the scaling relation between osmotic pressure and density for a compressed brush can be determined from the equation of state of bulk polymers.^[70] In our second method to predict the force on particles in brushes (the Bulk method), we employ and extrapolate this observation and obtain the functional dependence of both Π^B and γ^B on the monomer density ρ from simulations of 6075 polymers (degree of polymerization $N = 32$) in athermal, implicit solvent moving freely in a box with periodic boundaries in x and y and two mathematical walls (9-3 LJ, $\epsilon = 1$ and $\sigma = 1$) positioned 96σ apart at the top and the bottom of the box in z . We tested that this distance between the walls is large enough such that our pressure is not influenced by finite-size and interfacial effects within the statistical error. All other parameters are the same as in the brush simulations.

The osmotic pressure for different densities between $\rho = 0.05 \sigma^{-3}$ and $\rho = 0.4 \sigma^{-3}$ is calculated from the force on each wall (averaged over 4,000,000 timesteps (20000 τ)) divided by the area of the wall. The results are shown in Figure 5.2 (black symbols). When we fit the simulation data with a power law function $\Pi^B = c\rho^\kappa$ (orange line in Figure 5.2), we find that the osmotic pressure can be accurately described by $\Pi^B = 5.365\rho^{2.735}$ (R^2 : 0.9985). The exponent of $\kappa = 2.735$ is higher than the exponents proposed by mean field ($\kappa = 2$) and scaling ($\kappa = 9/4$) arguments,^[55] but is in qualitative agreement with the general observation that these approximations underestimate the pressure at higher densities.^[70-74]

The density near the wall $\rho(z)$ is also determined from the bulk simulations, and used in combination with Equation 5.6 to obtain $h(z)$ for each bulk density ρ , as shown in the inset of Figure 2B. Integration of $h(z)$ results in the $\hat{\Pi}^B$ for the different bulk densities, which we fitted with the function proposed by Louis et al.^[54], except that we do not impose that $-\hat{\Pi}^B \sim \rho^{-0.77}$ as expected from scaling theory^[55] for the semi-dilute regime and therefore we have an additional fitting parameter. This resulted in the following function:

$$\begin{aligned}\hat{\Pi}^B &= -1.074 R_g (1 + a\rho + b\rho^3)^c \\ \hat{\Pi}^B &= -1.074 \cdot 3.17 (1 + 5.443\rho + 35.52\rho^3)^{2.084}\end{aligned}\tag{5.11}$$

(R^2 : 0.9976), where a , b and c are fitting parameters and R_g is the radius of gyration of the bulk polymers ($R_g = 3.17 \sigma$).

In agreement with predictions from renormalization group theory, our function for $\hat{\Pi}^B$ equals $-1.074 R_g$ for $\rho \rightarrow 0$.^[54,75] The relations found for Π^B and $\hat{\Pi}^B$ can be inserted into Equation 5.4 to find the surface tension γ^B such that the relations for Π^B and γ^B can then be substituted into Equation 5.3 and corrected for shape and orientation by ξ_V in order to find the dependency of the inclusion free energy on the local monomer density.

Figure 5.2: a) The osmotic pressure (Π) as a function of the density (ρ), Orange lines: fit with functions described in the text; Black points: data extracted from simulations, inset: schematic representation of the simulation used for the Bulk Method. b) Relative adsorption (Γ) versus the density, inset: Relative density $h(z)$ as a function of the distance from the wall at bulk densities of 0.1 (light grey line) to 0.4 (black line) σ^{-3} .

5.3 Results and discussion

To explain the typical shape of a force-distance curve for a particle in a brush, we show in Figure 5.3 the average monomer number-density ρ (orange line) and the theoretical force F (calculated using Equation 5.10 and the local monomer density) on an ideal spherical particle of radius $r = 2 \sigma$ (black line) as a function of the distance from the anchoring surface D . Far from the surface ($D > 60 \sigma$), $\rho = 0$ and, consequently $F = 0$. For shallow indentation ($D = 58-60 \sigma$), the force slightly increases. Upon further indentation, the force strongly increases until it reaches a maximum at $D = 48.6 \sigma$. After the maximum, the force gradually decreases until it approaches values close to zero where the density is approximately constant (for $D < 15 \sigma$). The presence of a force-maximum near the top of the brush seems intuitively plausible, because, at these distances, the difference in density above and below the particle is the largest. Nevertheless, the inflection point of the density profile at $D = 52.1 \sigma$ (dashed orange line in Figure 5.3) is not at the same distance as the maximum in the force ($D = 48.6 \sigma$, dashed black line). There are two reasons for this: (1) the exponents describing the density-dependence of the inclusion free energy are different from one (see Equation 5.10), such that $d^2 E_{\text{incl}} / dD^2 \neq 0$ when $d^2 \rho / dD^2 = 0$, and (2) the inflection point of the *effective* density acting on our particle is shifted to lower distances compared to the inflection point of the actual density profile, due to averaging over the particle volume and the particle surface. Figure 5.3 also shows that close to the surface ($D < 4 \sigma$), there are 2-3 oscillations in the density (causing F to oscillate too). These oscillations are commonly observed in MD simulations of polymer brushes^[76-78] and caused by a symmetry-breaking effect of the wall, which induces layering.

Figure 5.3: Right axis, black line: Theoretical inclusion force F on a sphere of radius 2σ , at various distances D from the surface. Left axis, yellow line: density profile ρ at various distances D from the surface.

As expected, the shape of our density profile (orange line Figure 5.3) deviates from the uniform density distribution assumed in the Alexander-de Gennes model.^[43-44] However, our density distribution also deviates from the parabolic profile predicted by analytical self-consistent field theory (SCFT).^[50-52] The reason for this is that the parabolic profile should only hold for dilute brushes comprised of polymers of very large N , such that the stretching parameter $\beta_s \rightarrow \infty$ ($\beta_s = N(3w^2\sigma_g^2/2a^2)^{1/3}$, where w is the interaction parameter and a the Kuhn length)^[79]. This limit is hard to obtain in MD simulations, since the relaxation time of polymers in brushes strongly increases with the degree of polymerization^[80] ($\tau \sim N^{3.7}$) resulting in very large equilibration times for large N . Instead, we observe a rounded low-density region in the top of the brush or 'final blob' at $D = 58-60\sigma$. This rounded density-distribution is probably caused by thermal fluctuations in the concentration at the outer region^[81] and by the translational entropy of the polymer ends, which broadens its density distribution.^[82] Also, the density distribution deeper in the brush is flatter than the parabolic density profile, which is caused by our relatively high grafting density of 0.16 chains σ^{-2} . At these higher densities an effective saturation-effect comes into play,^[83-84] which would ultimately result in a block density-profile in the limit of a dense, closely packed monolayer.^[71,73]

Figure 5.4: The inclusion free energy E_{incl} versus the volume of the particle V upon deep inclusion ($D = 15 \sigma$) for the differently shaped particles (denoted by the different symbols) at different orientations (denoted by the different colors). The deviation from the linear fit through the data for the vertical rod, equals the correction factor ξ_v . The error bars denote the standard error of the mean for a 95% confidence interval.

The exact evolution of force vs distance data as depicted in Figure 5.3, depends on the shape and orientation of the inserted particle. We try to predict these force-curves with the two methods described in the Model and Methods section. To do so, we determine first the geometry- and orientation-dependent correction factor ξ_v of Kim and O'Shaughnessy^[23] Figure 5.4 shows the inclusion free energy E_{incl} for deep inclusion versus the volume of the particle V for all particles used in our simulations. E_{incl} is calculated by integration of the inclusion force F from $D = 15 \sigma$ to infinity. For deep inclusions, the excluded volume interactions dominate over surface tension effects^[30] such that E_{incl} for our particles is theoretically described by Equation 5.7. The vertically positioned rod will cause only small deviations in the polymer paths such that we can assume that $\xi_v = 1$ in these simulations.^[23] This allows us to determine the osmotic pressure at deep inclusion ($\Pi = 0.382 \epsilon \sigma^{-3}$), which defines the slope of the linear relation between E_{incl} and V without correction (orange line in Figure 5.4). For the other shapes and orientations, ξ_v can now be determined via $\xi_v = E_{\text{incl}}/(\Pi V)$.

The correction factors ξ_v derived from the data presented in Figure 5.4 are given in Table 5.1. In agreement with the correction factors predicted to be $1 < \xi_v < 1.5$ by Ref. ^[23], we find that $\xi_v = 1.27$ for the horizontally positioned rod, which, in contrast to the vertical rod, does alter the polymer paths in the brush. For the rod in a diagonal orientation, we find that $\xi_v = 1.15$, which is near the average of the ξ_v for the vertical and horizontal rod. The correction factors for the vertical discs ($\xi_v = 1.12$ and 1.11 for the small and the large disc, respectively) are relatively small compared to the ξ_v for the horizontal rod. The reason for this might be that the vertical disc has a more streamlined, curved shape and that the projected area is approximately 3x smaller than for the rod. For the horizontal discs, we need again a larger correction factor ($\xi_v = 1.23$ and 1.27 for the small and the large disc, respectively), because the projected area is 3x larger. The ξ_v for the cubes

are relatively small, because of their more streamlined shape. For the small cube that is oriented face-down, the correction is slightly smaller $\xi_v = 1.08$ than for the small cube oriented with one of its corners down ($\xi_v = 1.12$), which is most likely because its in-plane diameter is $\sqrt{3}$ smaller, though the effect of the smaller in-plane area is partly compensated by its less-streamlined shape. For the larger cubes, we find the same correction-factor for both orientations ($\xi_v = 1.05$). The uncertainty in the latter correction-factors is, however, larger than for the other correction factors, because the inclusion force for the large cubes at $D = 15 \sigma$ is already slightly influenced by the anchoring surface (see also Figure 5.5C and D). Kim and O'Shaughnessy^[23] predicted that the correction factor for a sphere should be $1 < \xi_v < 1.25$. This is in agreement with our finding that $\xi_v = 1.21$ for the large sphere and $\xi_v = 1.18$ for the small sphere. Note that the size-dependence of the correction factor (variations of 2-3%) is much smaller than shape- and orientation-dependence of the correction factor (variations of 10-30%). To test if these correction factors are transferrable to systems of different grafting densities, we performed simulations of the vertical cylinder and the small sphere in a brush of higher grafting density (σ_g of 0.21 chains σ^{-2}) than our default grafting density. In these simulations we found that the correction factor is the same within the stochastic error ($\xi_v = 1.19 \pm 0.01$ for $\sigma_g = 0.21$ chains σ^{-2} compared to $\xi_v = 1.18 \pm 0.00$ for $\sigma_g = 0.16$ chains σ^{-2}).

With the obtained correction factors and the relations between the local monomer density and the osmotic pressure / relative adsorption derived via the SC method and the Bulk method, we can compute the predicted force curves from the height-dependent local monomer density in the brush for each particle and at each orientation. For the Bulk method, the forces are directly calculated. For the SC method the deviation of the calculated forces (Equation 5.10) from the forces extracted from the simulations is minimized for all particles and orientations simultaneously using the function `fminsearch` as implemented in Matlab. The resulting fitting parameters for the prefactors in Equation 5.10 are $\alpha = 3.2$ and $\beta = -0.055$. De Gennes^[55] reasoned that α should be close to one for polymers free in solutions. Our larger α might be an effect of the constraint on the polymer ends^[85] or a compensation for the underestimation of the increase in osmotic pressure with density in the scaling arguments.^[70-74]

The predicted forces and the forces extracted from the simulations are shown in Figure 5.5 for all shapes and orientations of the particles. The data points (crosses) give the forces extracted from the simulations, while the calculated force is represented by the dashed lines for the Bulk method and the full lines for the SC method. The agreement between calculation and simulation is quantified by R^2 , which equals one minus the sum of squares of residuals normalized by the total sum of squares (such that $R^2 = 1$ for a perfect agreement). The R^2 for our results between $D = 10 \sigma$ and $D = 60 \sigma$ on the different particle-shapes and orientations are given in the figure caption of Figure 5.5. The SC

method (average $R^2 = 0.83$) performs better than the Bulk method (average $R^2 = 0.70$). By way of comparison, when using a model based on only the excluded volume interaction (First term in Equation 5.3), we got $R^2 = 0.72$ for the SC method and when using a model based on only the excluded volume interactions and the correction factor ξ_V , we obtained $R^2 = 0.79$ for the SC method. This shows that we find the best agreement between theory and simulations for the full model, which includes the excluded volume interactions, the surface tension and shape and orientation dependent correction factor.

Figure 5.5: The force F vs distance D data for the different particle-shapes and orientations. The symbols (crosses) represent the forces extracted from the simulations. The dashed lines give the calculated force curves using the Bulk method and the full lines give the fitted force curves using the SC method. (A) Sphere of diameter 5σ (orange, SC $R^2=0.87$, Bulk $R^2=0.82$) and 8σ (black, SC $R^2=0.84$, Bulk $R^2=0.88$). (B) Rod in horizontal (black, SC $R^2=0.87$, Bulk $R^2=0.88$), diagonal (grey, SC $R^2=0.81$, Bulk $R^2=0.58$) and vertical (orange, SC $R^2=0.61$, Bulk $R^2=-0.48$) orientation. (C) Cube oriented face down of edge-length 6σ (orange, SC $R^2=0.92$, Bulk $R^2=0.87$) and 8σ (black, SC $R^2=0.87$, Bulk $R^2=0.84$). (D) Cube oriented point down of edge-length 6σ (orange, SC $R^2=0.89$, Bulk $R^2=0.86$) and 8σ (black, SC $R^2=0.87$, Bulk $R^2=0.88$). (E) Disc in horizontal orientation of diameter 7σ (orange, SC $R^2=0.77$, Bulk $R^2=0.86$) and 9σ (black, SC $R^2=0.81$, Bulk $R^2=0.91$). (F) Disc in vertical orientation of diameter 7σ (orange, SC $R^2=0.83$, Bulk $R^2=0.62$) and 9σ (black, SC $R^2=0.85$, Bulk $R^2=0.65$).

Figure 5.5A shows the force-distance curves for the spheres of diameter $d = 5 \sigma$ (orange data) and $d = 8 \sigma$ (black data). In agreement with the theoretical prediction, the maximum force is approximately 3 times larger for the particle of $d = 8 \sigma$ compared to the sphere of $d = 5 \sigma$. The distance at which we observe the maximum in the force shifts to a slightly lower value for the larger sphere (for $d = 5 \sigma$ at $D = 48 \sigma$ and for $d = 8 \sigma$ at $D = 47 \sigma$). This effect has been observed before for spherical nanoparticles in theta-solvent,^[33] and is caused by a down-ward shift in the inflection point of the effective density that acts on the total volume and surface of the particle. For the rods in Figure 5.5B, we observe a similar trend: For the horizontal rod (black data points), where the entire volume is close to the center of mass position in D , the peak-position is found at the largest distance ($D = 47 \sigma$). For the diagonal rod (grey data), the force-maximum is situated at $D = 46 \sigma$ and for the vertical rod (orange data), we observed a force-maximum at $D = 45 \sigma$. For the horizontal rod, we also observe the sharpest peak, because the effective density is averaged over the smallest height. For the cubes, the forces are almost independent on the orientation (compare Figure 5.5C and D). The maximum force for both orientations is approximately 2 times higher for the large cube (black data) compared to the small cube (orange data), because the volume and the surface are approximately 2 times larger. As for the spheres, the peak-positions for the smaller cubes are at approximately 2% larger distances from the surfaces. The discs (Figure 5.5E and F) show a similar effect of the particle orientation as the rod. For the horizontal discs, the position of the force maximum is approximately at 1% larger distances from the surface than for the vertical discs. For the larger discs, the maximum force is around 1.5 times higher for the disc of width 9σ compared to the disc of width 7σ . By integration of the force curves of Figure 5.5, the inclusion energy can be calculated, which can be used to compare the ease of particle-penetration into the brush. To do this, one should integrate from the D of a fixed force until infinity such that the inclusion energy at a fixed force can be compared.

Though both methods capture all qualitative features and the size-, shape- and orientation-dependent trends of the distance-dependent inclusion force, only the SC method comes close to a quantitative agreement. The agreement is, however, still not perfect. Especially for the vertical disc ($R^2 = 0.8$) and the vertical rod ($R^2 = 0.6$) the agreement is rather poor. We attribute these deviations between the calculated forces and the forces extracted from the simulations to a curvature effect on the surface tension.^[54] In our estimation of the interfacial energy, we assumed that the surface was locally flat. This assumption might break down for geometries with locally large curvatures. Also, due to too much stochastic noise, we have been unable to determine shape- and orientation-dependent correction factors ξ_s for the interfacial energy term in Equation 5.3. Including such a correction factor would improve the agreement between theory and simulation.

The Bulk method overestimates the forces by 20-30% for all shapes and orientations. A qualitatively similar overestimation has been observed in model calculations for brushes under mechanical confinement^[86] based on the osmotic pressure of bulk polymers. However, in contrast to our relatively small deviations, the deviations in these systems were found to be close to two orders of magnitude. Therefore, we think our overestimation might be of a different origin. In our theoretical calculation, we assumed that the particles consist of hard spheres, while in our simulations we used the LJ potential. The softer LJ potential might reduce the effective excluded volume compared to the theoretical excluded volume, such that inclusion energies and forces in the simulations would be smaller than in theory.

We note that the model discussed in the present study only holds for repulsive particle-polymer interactions. These conditions are met when the particle-solvent and polymer-solvent interactions are stronger than the particle-polymer interactions such that the interaction between the particles and polymers is effectively repulsive. When the particle-polymer interactions are effectively attractive, additional contributions to the inclusion free energy need to be incorporated.

Though our study of the inclusion of non-spherical particles in polymer brushes is a significant step towards modeling more realistic systems, our system is still idealized and it does not capture all features and conditions found in applications. The brushes prepared in a laboratory environment with techniques such as surface-initiated atom-transfer radical polymerization can have a polydispersity index between 1.5 and 2,^[87] while our polymers are monodisperse. Polydispersity will change the density profile of the brush^[88] to a more linear or even concave shape^[89] and for small particles this will reduce the inclusion free energy^[25] and the height of the maximum in the force-distance curve^[33] making it easier for particles to penetrate into the brush. Another idealization is that our system was kept in (thermal) equilibrium, while in most applications the particles and brushes are in relative motion, which is imposed by movement of the walls or external solvent flow. The induced shear-stress on the polymers will tilt them and densify the brushes^[90] such that the density distribution changes towards a block profile. The latter causes the maximum in the force-distance curve to be higher and sharper. Another consequence of shear-stress is cyclic motion of the polymer ends,^[91] which might enhance mixing between brushes and particles. We intend to address these issues in future studies.

5.4 Conclusions

In summary, we have shown in a MD simulation study that the forces on cubes, rods, discs and spheres, that are included in polymer brushes at different orientations and different distances from the anchoring surface, can be described by a model that incorporates three effects. The first effect is that the particle-induced cavity in the brush

results in an excluded volume interaction. Secondly, there is an energy penalty for the created particle-polymer interface. Thirdly, the polymers of brushes will follow a streamlined path around the particles such that the pressure-gradient over the surface will differ from the gradient in a polymer solution or melt. The latter can be captured in a correction factor for the inclusion free energy due to the excluded volume interaction. This model describes the dependency of the inclusion force on the osmotic pressure and the effective adsorption at the particle-polymer interface. By relating the osmotic pressure and the effective adsorption to the local density using scaling arguments of de Gennes, we find close to quantitative agreement between theory and simulations.

5.5 References

- [1] J. Perez-Vilar, R. L. Hill, *J. Biol. Chem.* **1999**, 274 (45), 31751-31754 | 10.1074/jbc.274.45.31751
- [2] B. S. Turner, R. Bansil, *Current Opinion in Colloid and Interface Science* **2006**, 11 (2?3), 164 - 170 | 10.1016/j.cocis.2005.11.001
- [3] D. J. Thornton, K. Rousseau, M. A. McGuckin, *Annu. Rev. Physiol.* **2008**, 70 (1), 459-486 | 10.1146/annurev.physiol.70.113006.100702
- [4] A. C. Balazs, T. Emrick, T. P. Russell, *Science* **2006**, 314, 1107-1110 | 10.1126/science.1130557
- [5] F. Costantini, E. M. Benetti, D. N. Reinhoudt, J. Huskens, G. J. Vancso, W. Verboom, *Lab on a chip* **2010**, 10, 3407-12 | 10.1039/c0lc00187b
- [6] J. Huang, Y. Wang, M. Laradji, *Macromolecules* **2006**, 39 (16), 5546-5554 | 10.1021/ma060628f
- [7] O. Ozay, S. Ekici, Y. Baran, N. Aktas, N. Sahiner, *Water Res.* **2009**, 43 (17), 4403 - 4411 | 10.1016/j.watres.2009.06.058
- [8] H. Sun, S. Ma, Y. Yuan, Q. Cao, *Journal of Macromolecular Science Part B-Physics* **2013**, 52 (May 2015), 852-860 | 10.1080/00222348.2012.738153
- [9] M. Klein Gunnewiek, E. M. Benetti, A. Di Luca, C. A. van Blitterswijk, L. Moroni, G. J. Vancso, *Langmuir* **2013**, 29 (45), 13843-52 | 10.1021/la403360r
- [10] I. Tokareva, S. Minko, J. H. Fendler, E. Hutter, *J. Am. Chem. Soc.* **2004**, 126 (49), 15950 | 10.1021/ja044575y
- [11] H. Merlitz, G.-L. He, C.-X. Wu, J.-U. Sommer, *Phys. Rev. Lett.* **2009**, 102 (11), 115702 | 10.1103/Physrevlett.102.115702
- [12] Y. Yu, B. D. Kieviet, E. Kutnyanszky, G. J. Vancso, S. de Beer, *ACS Macro Lett.* **2015**, 4 (1), 75 | 10.1021/mz500775w
- [13] L. Dos Ramos, S. de Beer, M. A. Hempenius, G. J. Vancso, *Langmuir* **2015**, 31 (23), 6343-6350 | 10.1021/acs.langmuir.5b01105
- [14] L. Spirin, A. Galuschko, T. Kreer, *Macromolecules* **2011**, 44 (23), 9399 | 10.1021/ma2014029
- [15] S. Krishnan, C. J. Weinman, C. K. Ober, *J. Mater. Chem.* **2008**, 18, 3405-3413 | 10.1039/B801491D
- [16] A. Halperin, M. Kröger, *Macromolecules* **2011**, 44 (17), 6986-7005 | 10.1021/ma201006h
- [17] X. Zhu, D. Janczewski, S. S. C. Lee, S. L.-M. Teo, G. J. Vancso, *ACS applied materials & interfaces* **2013**, 5 (13), 5961-5968 | 10.1021/am4015549
- [18] M. L. W. Knetsch, L. H. Koole, *Polymers* **2011**, 3, 340 | 10.3390/polym3010340

- [19] B. D. Kieviet, P. M. Schön, G. J. Vancso, *Lab on a chip* **2014**, *14*, 4159-4170 | 10.1039/c4lc00784k
- [20] I. V. Neratova, T. Kreer, J.-U. Sommer, *Macromolecules* **2015**, *48* (11), 3756-3766 | 10.1021/acs.macromol.5b00042
- [21] M. E. V. Johansson, D. Ambort, T. Pelaseyed, A. Schütte, J. K. Gustafsson, A. Ermund, D. B. Subramani, J. M. Holmén-Larsson, K. A. Thomsson, J. H. Bergström, S. Van Der Post, A. M. Rodriguez-Piñeiro, H. Sjövall, M. Bäckström, G. C. Hansson, *Cell. Mol. Life Sci.* **2011**, *68*, 3635-3641 | 10.1007/s00018-011-0822-3
- [22] G. Subramanian, D. R. M. Williams, P. A. Pincus, *Macromolecules* **1996**, *29* (11), 4045-4050 | 10.1021/Ma946439r
- [23] J. U. Kim, B. O'Shaughnessy, *Macromolecules* **2006**, *39* (1), 413-425 | 10.1021/ma050817i
- [24] J. U. Kim, M. W. Matsen, *Macromolecules* **2008**, *41* (1), 246-252 | 10.1021/ma071906t
- [25] W. M. de Vos, F. A. M. Leermakers, A. de Keizer, J. M. Kleijn, M. A. Cohen Stuart, *Macromolecules* **2009**, *42* (15), 5881-5891 | 10.1021/ma900819b
- [26] L. M. Hall, K. S. Schweizer, *Soft Matter* **2010**, *6* (5), 1015 | 10.1039/b919160g
- [27] A. Halperin, M. Kröger, E. B. Zhulina, *Macromolecules* **2011**, *44* (9), 3622-3638 | 10.1021/ma200068d
- [28] K. H. A. Lau, C. Ren, S. H. Park, I. Szleifer, P. B. Messersmith, *Langmuir* **2012**, *28* (4), 2288-2298 | 10.1021/la203905g
- [29] S. A. Egorov, *J. Chem. Phys.* **2012**, *137* (May), | 10.1063/1.4757017
- [30] A. Milchev, D. I. Dimitrov, K. Binder, *Polymer* **2008**, *49* (17), 3611-3618 | 10.1016/j.polymer.2008.04.032
- [31] J. Yaneva, D. I. Dimitrov, A. Milchev, K. Binder, *J. Colloid Interface Sci.* **2009**, *336* (1), 51-8 | 10.1016/j.jcis.2009.03.062
- [32] V. Ermilov, A. Lazutin, A. Halperin, *Macromolecules* **2010**, *43* (7), 3511-3520 | 10.1021/ma9027273
- [33] H. Merlitz, C.-X. Wu, J.-U. Sommer, *Macromolecules* **2012**, *45* (20), 8494-8501 | 10.1021/ma301781b
- [34] T. Curk, F. J. Martinez-Veracoechea, D. Frenkel, J. Dobnikar, *Soft Matter* **2013**, *9* (23), 5565 | 10.1039/c3sm50486g
- [35] M. G. Opferman, R. D. Coalson, D. Jasnow, A. Zilman, *Langmuir* **2013**, *29* (27), 8584-8591 | 10.1021/la4013922
- [36] T. Curk, F. J. Martinez-Veracoechea, D. Frenkel, J. Dobnikar, *Nano Lett.* **2014**, *14* (5), 2617-2622 | 10.1021/nl500449x
- [37] J. Cheng, A. Vishnyakov, A. V. Neimark, *The Journal of Chemical Physics* **2015**, *142*, 34705 | 10.1063/1.4905894
- [38] R. A. Gage, E. P. K. Currie, M. A. Cohen Stuart, *Macromolecules* **2001**, *34* (15), 5078-5080 | 10.1021/ma0018695
- [39] S. Santer, J. Rühle, *Polymer* **2004**, *45* (25), 8279 - 8297 | 10.1016/j.polymer.2004.09.085
- [40] E. Filippidi, V. Michailidou, B. Loppinet, J. Rühle, G. Fytas, *Langmuir* **2007**, *23* (13), 5139-5142 | 10.1021/la0637162
- [41] I. Luzinov, S. Minko, V. V. Tsukruk, *Soft Matter* **2008**, *4*, 714-725 | 10.1039/B718999K
- [42] R. Oren, Z. Liang, J. S. Barnard, S. C. Warren, U. Wiesner, W. T. S. Huck, *J. Am. Chem. Soc.* **2009**, *131* (5), 1670-1671 | 10.1021/ja8090092

- [43] S. Alexander, *J. Phys.-Paris* **1977**, 38 (8), 983 | 10.1051/jphys:01977003808098300
- [44] P. G. de Gennes, *Macromolecules* **1980**, 13, 1069 | 10.1021/ma60077a009
- [45] S. T. Milner, *Science* **1991**, 251 (4996), 905 | 10.1126/science.251.4996.905
- [46] M. Chen, W. H. Briscoe, S. P. Armes, J. Klein, *Science* **2009**, 323 (5922), 1698 | 10.1126/science.1169399
- [47] S. de Beer, E. Kutnyanszky, P. M. Schön, G. J. Vancso, M. H. Müser, *Nat. Commun.* **2014**, 5, 3781 | 10.1038/ncomms4781
- [48] J. F. Stanislaw, *Rheol. Acta* **1982**, 565 (4-5), 564 | 10.1007/Bf01534348
- [49] J. U. Kim, B. O'Shaughnessy, *Phys. Rev. Lett.* **2002**, 89, 238301 | 10.1103/PhysRevLett.89.238301
- [50] A. N. Semenov, *Sov. Phys. JETP* **1985**, 61 (4), 733-742 |
- [51] S. T. Milner, T. A. Witten, M. E. Cates, *Macromolecules* **1988**, 21 (8), 2610 | 10.1021/Ma00186a051
- [52] A. M. Skvortsov, A. A. Gorbunov, I. V. Pawlushkov, Y. B. Zhulina, O. V. Borisov, V. A. Pryamitsyn, *Polym. Sci. USSR* **1988**, 30, 1706-1715 | 10.1016/0032-3950(88)90393-0
- [53] D. Zhang, J. Cheng, Y. Jiang, X. Wen, L. Zhang, *J. Polym. Sci., Part B: Polym. Phys.* **2013**, 51 (6), 392-402 | 10.1002/polb.23224
- [54] A. A. Louis, P. G. Bolhuis, E. J. Meijer, J. P. Hansen, *J. Chem. Phys.* **2002**, 116 (1), 10547-10556 | 10.1063/1.1473658
- [55] P. G. de Gennes, *Scaling Concepts in Polymer Physics*. Cornell University Press: **1979**.
- [56] G. S. Grest, K. Kremer, *Phys. Rev. A* **1986**, 33 (5), 3628 | 10.1103/PhysRevA.33.3628
- [57] K. Kremer, G. S. Grest, *The Journal of Chemical Physics* **1990**, 92 (8), 5057 | 10.1063/1.458541
- [58] D. Mukherji, M. H. Müser, *Macromolecules* **2007**, 40 (5), 1754-1762 | 10.1021/ma0627370
- [59] S. de Beer, M. H. Müser, *Macromolecules* **2014**, 47 (21), 7666-7673 | 10.1021/ma501718b
- [60] W. J. Brittain, S. Minko, *Polym. Sci., Part A: Polym. Chem.* **2007**, 45 (16), 3505 | 10.1002/pola.22180
- [61] B. Zdyrko, V. Klep, I. Luzinov, *Langmuir* **2003**, 19 (24), 10179-10187 | 10.1021/la034974r
- [62] R. Descas, J.-U. Sommer, A. Blumen, *Macromol. Theory Simul.* **2008**, 17 (9), 429 | 10.1002/mats.200800046
- [63] W. Humphrey, A. Dalke, K. Schulten, *J. Molec. Graphics* **1996**, 14 (1), 33 | 10.1016/0263-7855(96)00018-5
- [64] S. Plimpton, *J. Comp. Phys.* **1995**, 117 (1), 1 | 10.1006/jcph.1995.1039
- [65] A. Halperin, E. B. Zhulina, *Langmuir* **2010**, 26 (22), 8933-8940 | 10.1021/la9047374
- [66] W. K. den Otter, W. J. Briels, *The Journal of Chemical Physics* **1998**, 109, 4139-4146 | 10.1063/1.477019
- [67] J. S. Rowlinson, B. Widom, *Molecular Theory of Capillarity*. Oxford University Press: Oxford, **1989**.
- [68] O. A. Guskova, S. Pal, C. Seidel, *EPL (Europhysics Letters)* **2009**, 88 (3), 38006 | 10.1209/0295-5075/88/38006
- [69] D. Marsh, *Biophys. J.* **2004**, 86 (April), 2630-2633 | 10.1016/S0006-3495(04)74317-9

- [70] S. M. Balko, T. Kreer, P. J. Costanzo, T. E. Patten, A. Johnner, T. L. Kuhl, C. M. Marques, *PLoS ONE* **2013**, 8, e58392 | 10.1371/journal.pone.0058392.t001
- [71] D. F. K. Shim, M. E. Cates, *J. Phys. France* **1989**, 50 (24), 3535-3551 | 10.1051/jphys:0198900500240353500
- [72] M. D. Whitmore, R. Baranowski, *Macromol. Theory Simul.* **2005**, 14 (2), 75-95 | 10.1002/mats.200400104
- [73] P. M. Biesheuvel, W. M. de Vos, V. M. Amoskov, *Macromolecules* **2008**, 41 (16), 6254-6259 | 10.1021/ma800828c
- [74] T. Kreer, S. M. Balko, *ACS Macro Lett.* **2013**, 2, 944-947 | 10.1021/mz4004387
- [75] A. Hanke, E. Eisenriegler, S. Dietrich, *Phys. Rev. E* **1999**, 59, 6853-6878 | 10.1103/PhysRevE.59.6853
- [76] G. S. Grest, M. Murat, *Macromolecules* **1993**, 26 (12), 3108-3117 | 10.1021/ma00064a019
- [77] D. I. Dimitrov, A. Milchev, K. Binder, *The Journal of Chemical Physics* **2007**, 127, 84905 | 10.1063/1.2768525
- [78] S. de Beer, *Langmuir* **2014**, 30, 8085-8090 | 10.1021/la5013473
- [79] R. R. Netz, M. Schick, *Macromolecules* **1998**, 31 (15), 5105-5122 | 10.1021/ma9717505
- [80] D. Reith, A. Milchev, P. Virnau, K. Binder, *Macromolecules* **2012**, 45 (10), 4381-4393 | 10.1021/ma202745b
- [81] J. Wittmer, A. Johnner, J. F. Joanny, K. Binder, *The Journal of Chemical Physics* **1994**, 101, 4379-4390 | 10.1063/1.468410
- [82] J. U. Kim, M. W. Matsen, *The European Physical Journal E* **2007**, 23, 135-144 | 10.1140/epje/i2007-10188-1
- [83] I. Coluzza, J.-P. Hansen, *Phys. Rev. Lett.* **2008**, 100, 16104 | 10.1103/PhysRevLett.100.016104
- [84] I. G. Elliott, T. L. Kuhl, R. Faller, *Macromolecules* **2010**, 43 (21), 9131-9138 | 10.1021/ma101252c
- [85] P. L. Hansen, J. A. Cohen, R. Podgornik, V. A. Parsegian, *Biophys. J.* **2003**, 84 (1), 350 - 355 | 10.1016/S0006-3495(03)74855-3
- [86] S. B. Abbott, W. M. de Vos, L. L. E. Mears, B. Cattoz, M. W. A. Skoda, R. Barker, R. M. Richardson, S. W. Prescott, *Macromolecules* **2015**, 48 (7), 2224-2234 | 10.1021/ma502246r
- [87] C. Kang, R. M. Crockett, N. D. Spencer, *Macromolecules* **2014**, 47 (1), 269-275 | 10.1021/ma401951w
- [88] N. Dan, M. Tirrell, *Macromolecules* **1993**, 26 (24), 6467-6473 | 10.1021/Ma00076a024
- [89] W. M. de Vos, F. A. M. Leermakers, *Polymer* **2009**, 50 (1), 305 - 316 | 10.1016/j.polymer.2008.10.025
- [90] A. Milchev, D. I. Dimitrov, K. Binder, *Biomechanics* **2010**, 4, 32202 | 10.1063/1.3396446
- [91] M. Müller, C. Pastorino, *EPL (Europhysics Letters)* **2008**, 81 (2), 28002 | 10.1209/0295-5075/81/28002

Optimal shaking conditions for particle-absorption in swollen polymer brushes

Particles in polymer brushes have attracted great interest due to their ability to closely mimic biological systems. By employing molecular dynamics, the change in particle absorption by polymer brushes was studied when the brush-substrate is oscillated normal to the substrate. The effect of a moving brush-substrate on the increase of particle penetration depth can be up to 128%, for the sizes, periods and amplitudes studied. The results show that an optimum period and amplitude combination exists for which this increase in penetration depth is maximum. These optimum conditions arise around the resonance frequency of the polymer brush, which depends on the driving amplitude.

The content of this chapter will be submitted as *Optimal shaking conditions for particle-absorption in swollen polymer brushes*, B.D. Kieviet, G.J. Vancso, S. de Beer, **2016**

6.1 Introduction

Polymer brushes, which are densely packed polymers with one chain-end tethered to a substrate,^[1] have received great interest due to their versatility and relevance in biological systems.^[2-5] Polymer brushes can be used as model systems for the study of more complex matter, such as the surface of the small intestine, since the mucus layer that is covering the inner wall of the small intestine consists of polysaccharides which can exhibit polymer brush-like behavior at the surface.^[6]

To study the characteristics of polymer brushes, molecular dynamics (MD) is very often employed, due to its ability to straightforwardly control design parameters and thus provide meaningful scaling relations in a relatively short time. Furthermore, MD gives insight in monomer-monomer and monomer-particle interplay across the length scales and at time scales ranging between femtoseconds and milliseconds. MD, among other simulation techniques,^[7] has greatly improved the theoretical understanding of both the intramolecular^[8] and the intermolecular dynamics^[9] of polymer brushes. Furthermore, by using MD simulations, solvent-brush interactions could be investigated^[10] as well as the mushroom-to-brush transition of polymer brushes.^[11]

These studies could be used to improve the understanding of the absorption of nutrients in the mucus of the small intestine. Nutrients have different size and shape, like cubic structure of salts, or the rod-like structure of many proteins. Therefore, of special interest for biological systems, yet also for catalysis^[12] and the development of tunable materials,^[13] is the interaction of particles with polymer brushes and insertion of particles in polymer brushes, which has been subjected to theoretical study,^[14-22] computer simulations^[23-29] and experiments.^[21,30-34] For example, Yaneva et al.^[35] have performed equilibrium MD simulations on particles in static polymer brushes. In agreement with theoretical predictions,^[36] they found a critical particle size b^* , which can be approximated by using: $b^* \approx 0.4\sigma_g^{-2/3}$,^[15] where σ_g is the grafting density. Below this b^* the particles can disperse freely in the polymer brush. Above this value, up to a value $b_{\max}$, the particles are penetrating only a certain distance δ . Particles above size $b_{\max}$ remain adsorbed in the low density region near the end of the brush. In the limit of dilute brushes at very large N these particles are expelled from the brush entirely. These particles are shown to form aggregates when exposed to a poor solvent,^[37] or could even be trapped inside the brush by solvent exchange.^[38] Moreover, if particle interaction is favored over brush-particle interaction, aggregation behavior and phase separation of particles within the brush can occur.^[39-40] The existence of this $b_{\max}$ is also shown by various experiments on brushes in contact with nanoparticles.^[41-43] However, all these studies focused on brushes in thermal equilibrium. In reality, the mucus in the small intestine is in motion due to the contracting and expanding of the intestine. What happens with the critical particle size, or, more specifically, with the depth that the particle penetrates into the brush, when the

substrate, to which the polymers are attached, is perturbed, is thus far only studied under shear, i.e. in the direction parallel to the substrate.^[44-48] In other words, how does the motion of the polymer brush normal to the substrate influence the absorption of particles? Understanding the effect of motion of particle absorption is of paramount importance in the understanding of particle uptake in brush-like biological environments, such as the small intestine, for improvement of, e.g., drug efficacy.

In this chapter, we present MD simulations of a polymer brush in contact with particles of various size, where the wall to which the brush is tethered to is set in motion. We report an increase in penetration depth δ as function of period and amplitude of the oscillation. Furthermore, the optimum period and frequency of particle absorption are closely related to the brush-resonance values. This study is a prelude for MD studies on polymer brushes tethered to oscillating microchannels, to model the uptake of nutrients or drugs in the small intestine.

6.2 Model and methods

In the MD simulations, a generic coarse-grained model was employed, as first described by Grest and Kremer.^[49] This model was chosen due to its success in modeling polymer brushes.^[25] Furthermore, it allows comparison of this work with other work done on polymer brushes.^[35,50] The MD simulations were performed in the LAMMPS package.^[51] All interactions, except for consecutive monomer-monomer interactions, were governed by the Lennard-Jones (LJ) potential:^[52]

$$V_{LJ} = 4\epsilon \left(\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right) + \epsilon \quad (r < r_{cut}), \quad (6.1)$$

in which ϵ is the potential depth, σ the distance at which $V_{LJ} = 0$, and r_{ij} is the distance between two interacting particles. All interactions were repulsive to implicitly simulate a good solvent, in which the polymers are effectively repulsive. To do so, r_{cut} was set to $2^{1/6} \sigma$ and V_{LJ} was shifted upwards by ϵ .^[53] Throughout this work, ϵ and σ were set to 1. Consecutive monomer-monomer interactions were governed by the finitely extensible nonlinear elastic potential:

$$V_F = -0.5kR_0^2 \ln \left(1 - \left(\frac{r_{ij}}{R_0} \right)^2 \right) + 4\epsilon \left(\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{ij}} \right)^6 \right) + \epsilon. \quad (6.2)$$

To ensure that no bond-crossing occurred, R_0 was set to 1.5σ , and k was set to $30 \epsilon \sigma^{-2}$.^[49] Temperature control was regulated by a dissipative particle dynamics thermostat,^[54] since this thermostat correctly takes solvent hydrodynamics into account for implicit solvent simulations.^[55] The thermostat was set to $T = 1 \epsilon k_B^{-1}$, with the damping factor, γ , set to $0.5 m \tau^{-1}$.

We modelled a polymer brush by end-grafting 850 chains of 100 monomer beads of size 1σ onto a surface ($87.6 \sigma \times 103.9 \sigma$) at a grafting density, σ_g , of $0.093 \text{ chains } \sigma^{-2}$, which is 16x the critical grafting density. The substrate consists of a single layer of atoms, which

are bound to sites of a hexagonal lattice via harmonic springs ($k_{\text{lat}} = 16 \epsilon \sigma^{-2}$), and to their neighbors via anharmonic springs ($k_{\text{ww}} = 30 \epsilon \sigma^{-2}$). 500 particles were inserted in the system above the brush, where initially a mathematical constraint stopped the particles from entering the area where the brush resided and the whole system was allowed to equilibrate for 2500 τ . A schematical representation of both the polymer-brush particle system and the experiments performed can be found in Figure 6.1.

Figure 6.1: (a) Graphical representation of the polymer brush with particles. Rendered using Visual Molecular Dynamics.^[56] Particles are increased in size for clarity. (b) Schematic drawing of the simulation performed: The wall to which the polymer is tethered is oscillated, and the penetration depth δ is measured via the polymer and particle densities.

In this chapter, the critical particle radius b^* that can be expected was calculated as follows. From the self-consistent field theory calculations of Kim and O'Shaughnessy^[15] the following relation for b^* is found:

$$b^* = \frac{1}{\pi} \left(\frac{2aR_0^4}{h^2} \right)^{1/3}, \quad (6.3)$$

with a the monomer size, h the height of the brush and R_0 the Gaussian radius. However, this relation for b^* is for dry polymer brushes, hence, following Guskova et al.^[37], the Gaussian radius $R_0 = aN^{1/2}$ is replaced by the Flory radius $R_F = \alpha a N^{3/5}$, with α a solvent-dependent prefactor:

$$b^* = \frac{1}{\pi} \left(\frac{2aR_F^4}{h^2} \right)^{1/3} = 2^{1/3} \pi^{-1} N^{4/5} a \left(\frac{\alpha^2 a}{h} \right)^{2/3}. \quad (6.4)$$

When inserting the values for the polymer brush used: $a = 1$, $\alpha = 1.15$, $N = 100$ and using the first moment of the density profile as height:^[57] $h = \int z\phi_b dz / \int \phi_b dz = 35 \sigma$: $b^* = 1.88 \sigma$. Similarly, $b_{\max} = (1/2hb^{*3})^{1/4} = 3.29 \sigma$. The solvent dependent prefactor α was obtained via MD simulations from which the radius of gyration R_g was determined for free polymer chains of various length N . Variation of particle size was implemented into the MD simulations using a distance shift between $\sigma' = 0.8$ and 1.7σ in the LJ potential:

$$V_{\text{LJ}} = 4\epsilon \left(\left(\frac{\sigma}{r_{ij} - \sigma'} \right)^{12} - \left(\frac{\sigma}{r_{ij} - \sigma'} \right)^6 \right) + \epsilon \quad (r < 2^{1/6}\sigma + \sigma'). \quad (6.5)$$

This way only the interaction distance of the potential is changed, compared to simply changing the σ value of the LJ potential, the latter resulting in a 'softer' potential. The particle radius b is defined as the hard core radius: $b = \sigma + \sigma'$.

After the equilibration time, the mathematical constraint was removed and simultaneously the brush-surface was set to oscillate. The wall to which the polymers are tethered was oscillated by applying a sinusoidal function to the lattice sites of the substrate:

$$H_{\text{wall}}(t) = A_z \sin\left(\frac{2\pi}{P}t\right). \quad (6.6)$$

In the simulations the period P was varied from 70τ to 200τ in steps of 10τ , and the amplitude A_z was varied from 1σ to 10σ , in steps of 1σ . For ten oscillations, the particle density (ϕ_p) and the density of the monomers in the polymer brush (ϕ_b), averaged over the directions parallel to the brush-surface, were recorded every 0.25τ . To reduce the effects of the initial brush-particle contacts on the quantities of interest, and to allow the brush to equilibrate with this applied motion, the first five oscillations were not taken into account in the statistics (see below), which we tested to be long enough to reach a steady-state regime for both brush and particle dynamics. From the density profiles, ϕ_p and ϕ_b , the mean penetration depth δ was determined using:^[35]

$$\delta = \frac{1}{N} \sum_{t=5P}^{t=10P} \frac{\left[\int_0^\infty \phi_b(z, t) \phi_p(z, t) dz \right]^2}{\int_0^\infty \phi_b(z, t)^2 \phi_p(z, t)^2 dz}, \quad (6.7)$$

with an accuracy in z of 0.25σ . This definition of δ results in a length that is half the overlap length,^[35] as displayed in the inset of Figure 6.5.

To verify that the chosen starting conditions were not influencing the measurement, a different MD simulation was performed where the particles were already in contact with the brush when oscillating the substrate. Furthermore, a MD simulation was set-up where the mathematical boundary at distance 135σ was oscillated in sync with the substrate. For both tests, within the experimental error, no significant change was observed from the results presented here. At periods below 70τ and with $A_z > 6 \sigma$, a stable simulation could not be obtained.

6.3 Results and discussion

6.3.1 Shaking brushes without particles

The effect of the oscillating wall on the density profile can be seen in Figure 6.2.

Figure 6.2: (a) Density profile of the polymer brush, after n_p oscillations, at phase $\Phi = 0$. The wall (dashed line at 0σ) is oscillated with $A_z = 3$ and $P = 100$. After 6 oscillations ($n_p = 6$) the density profile reaches a steady state response. (b) First moment (FM) of the brush after n_p oscillations.

Figure 6.2 shows the density profile of the polymer brush, ϕ_b , while oscillating the surface, after n_p periods at phase $\Phi = 0$. The period and amplitude of the oscillation are 100τ and 3σ , respectively. The wall, to which the polymers are attached, is at 0σ (dashed line). The motion of the wall is started in the positive direction, i.e. in Figure 6.2 from left to right and back. After 5 periods, the density profile becomes stable ($<2\%$ deviation in first moment from $n_p = 10$), therefore, as mentioned above, only the last 5 periods are taken into account in further calculations. Furthermore, can be seen that the brush is first compressed up to $n_p = 4$, and then expands to a steady state height, which is about 10σ below the height at rest. Naturally, this effect is due to the substrate being in motion at $\Phi = 0$. To determine the relaxation time of the polymer brush system, the following experiment was performed: for the periods and amplitudes mentioned above and without particles, the wall was oscillated a single period. Afterwards, the polymer was left to equilibrate for 2500τ . The mean absolute value of the difference between the polymer density profile and the mean static polymer density profile was calculated over time:

$$\overline{|\Delta\rho(t)|} = \frac{1}{N} \sum_{z=0}^{\infty} |\phi_b(z, t) - \phi_0(z)|, \quad (6.8)$$

where $\phi_0(z)$ is the static density, determined by averaging the density of the static polymer brush for 2500τ . Subsequently, the maxima were fitted with an exponential decay by

$$\overline{|\Delta\rho(t)|} = A_0 e^{-\frac{t}{\tau_r}}, \quad (6.9)$$

where A_0 is the value of the mean absolute density difference at $t = 0$ and τ_r the relaxation time, see Figure 6.3a for both data (red line) and fit (black line) for $A_z = 5$ and $P = 100$. For reference, the mean absolute density difference for the static brush is also shown in Figure 6.3a (blue line).

Figure 6.3: Determination of relation time. a) Method on a single curve, at $P = 100 \tau$, $A_z = 5 \sigma$. b) Distribution of the relaxation time, for $P = 70$ to $P = 200 \tau$ and $A_z = 1$ to $A_z = 10 \sigma$.

The absolute value is used because the mean of the density difference is always zero, because the number of monomers in the system does not change. Figure 6.3b shows the distribution of the relaxation time, for the periods 70 to 200 τ and amplitudes 1 to 10 σ . From this, the relaxation time was found to be 253 τ .

As can be seen in Figure 6.2, the height of the polymer brush changes when moving the substrate. One can wonder if there is an optimum frequency at which the brush oscillated. Figure 6.4 shows the amplitude of the first moment of the brush over one period, at $n_p = 10$, as function of applied frequency (P^{-1}) and amplitude A_z . The first moment of the density is used as measure for the height of the brush (see Model and methods section).

Figure 6.4: Amplitude in first moment for $n_p = 10$ as function of frequency and A_z .

From Figure 6.4 can be concluded that there indeed is an optimum frequency to drive the brush. Surprisingly, this frequency is directly coupled to the amplitude of the oscillation. In contrast, for a harmonic oscillator, the resonance frequency is independent of the amplitude. At $A_z > 5$, the optimum frequency falls outside of the frequencies applied in this work, $f_{opt} > 70^{-1} \tau^{-1}$.

6.3.2 Static measurements with particles

To verify the system with the literature^[35] and to set a base-line for the subsequent measurements, the particle penetration depth was first evaluated without oscillation, or $A_z = 0$, which is shown in Figure 6.5.

Figure 6.5: Penetration depth δ as function of particle size b . b^* can be determined from the intersection of the fit lines. Inset: overlap density profiles ($\phi_b \cdot \phi_p$), from which δ is determined, for $b = 1, 1.5, 2$ and 3σ (black line, grey line and black and grey dashed line).

Figure 6.5 shows the penetration depth of particles in the brush. As mentioned in the Model and methods section, from theoretical calculations for swollen brushes in a good solvent^[37] $b^* = 1.88 \sigma$ and $b_{\max} = 3.29 \sigma$. From Figure 6.5, b^* could also be determined: the size from where the two fit lines intersect. Hence, for the oscillatory system, the size of the particles was varied from 1.8 to 2.7σ . The inset in Figure 6.5 shows three density overlap ($\phi_b \cdot \phi_p$) curves, via which the penetration depth δ is calculated, for particles of radius $1, 1.5, 2$ and 3σ in black, grey, black dashed and grey dashed respectively. The particles of $b = 1 \sigma$ and $b = 1.5 \sigma$ penetrate deep into the brush, the particles of $b = 2 \sigma$ are absorbed into the brush, but do not reach the substrate, whilst the particles of $b = 3 \sigma$ are expelled and only reside in the outer, low-density, layer of the brush..

6.3.3 Oscillatory measurements with particles

Oscillating the substrate to which the polymers are bound results in a cyclic response of the density profiles of both polymer brush and particles. In Figure 6.6, this effect is shown for a single period, at several intervals, from $\phi = 0$ to $\phi = 2\pi$.

Figure 6.6: Density profile of the polymer brush (black line, left axis) and particles (grey line, right axis) during a single period of oscillation. The profiles are lagging behind the oscillation of the wall (grey dashed line around 0σ) and both densities expand and contract in a synchronized way. For clarity, the particle density is averaged over 5 periods and 3σ at the same phase.

To illustrate the change in both density profiles in a single period of oscillation, both density profiles ϕ_b and ϕ_p , as function of phase, Φ , are shown in Figure 6.6. In this figure, $P = 100\tau$ and $A_z = 3\sigma$. For clarity, the density profile of the particles is averaged in z over a distance of 3σ and 5 periods. In case of the density profile of the brush this was unnecessary, and the accuracy in z of 0.25σ was retained. It can be seen that both profiles are lagging behind the oscillation of the wall (dashed grey line in Figure 6.6), and move in a synchronized way due to the hydrodynamics: when the brush is contracted, the particle profile is expanded and *vice versa*. From Figure 6.6, the effect of the

oscillation on the penetration depth is not directly clear. Therefore, Figure 6.7 shows the effect of oscillating the wall on both the density overlap and the penetration depth.

Figure 6.7: Effect of oscillating the wall on the density overlap (a) and penetration depth δ (b). In (a), solid lines: $P = 100 \tau$, dashed lines $P = 150 \tau$ and red lines $A_z = 3 \sigma$, black lines $A_z = 8 \sigma$. The static behavior is also shown (grey solid line). The particle size is 2.1σ (dashed line in (b)). In (b), open circles and crosses: oscillating wall, filled circles: static wall. Circles: $P = 100 \tau$, crosses $P = 150 \tau$. Colors as in (a). A clear increase in δ at $P = 100 \tau$ can be observed. Note that lowering the period increases δ more than raising the amplitude.

In Figure 6.7a, the effect of oscillating the wall on the density overlap ($\phi_b \cdot \phi_p$) is displayed. The increase in overlap due to the motion of the wall is evident. Several conclusions can be drawn from this figure: the motion of the wall results in a broadening of the density

overlap, due to a broadening of the brush density profile. Furthermore, both $P = 100 \tau$ and $P = 150 \tau$ at $A_z = 8 \sigma$ show particles penetrating the brush until the substrate, yet the density near the substrate is higher when $P = 100 \tau$. Interestingly, even the lower amplitude $A_z = 3 \sigma$ of $P = 100 \tau$ shows more particles deep in the brush than $A_z = 8 \sigma$ of $P = 150 \tau$. Hence, the period is of higher importance than the amplitude, where an increase in δ is concerned.

In Figure 6.7b, the penetration depth δ is shown for the same conditions as Figure 6.7a, with variation of size. The dashed line is size 2.1σ , which corresponds to the density overlap graphs in Figure 6.7a. Again, the increase in δ is clear. As mentioned before, effect on δ of the decrease in period P is more pronounced than the increase in amplitude A_z , as the increase in δ is higher for the conditions $P = 100 \tau$ and $A_z = 3 \sigma$ than for $P = 150 \tau$ and $A_z = 8 \sigma$, for example. Furthermore, for $P = 150 \tau$ and $A_z = 3 \sigma$, the increase in δ is almost insignificant. To show the effect of the moving wall on δ for all conditions studied, Figure 6.8 shows the δ increase, i.e., δ divided by the δ at $A_z = 0 \sigma$, for $P = 70$ to 200τ , $A_z = 1$ to 10σ and b equals 1.8 to 2.7σ .

Figure 6.8: Penetration depth ($\Delta\delta$) increase, normalized to the penetration depth at amplitude $A_z = 0 \sigma$, as function of size b , ranging from 1.8σ to 2.7σ and amplitude in z , ranging from 0σ to 10σ . The individual graphs are labeled according to the period applied, ranging from 70τ to 140τ .

Figure 6.8 shows the penetration depth increase $\Delta\delta$ of the particles of various size, as function of the amplitude of the oscillation in z . The increase is given in percentage points, and is normalized to the values at $A_z = 0$ of that certain particle size, as shown in Figure

6.5. The titles of the individual graphs are representing the period of the oscillation applied to the wall. As can be seen in Figure 6.8, the penetration-depth increase varies with amplitude, and is seemingly unaffected by the particle size.

From this can be concluded that, as expected, the particles penetrate deeper into the brush when a higher amplitude is applied. However, when the period is increased to 100τ , the increase in $\Delta\delta$ occurs at lower amplitudes. When increasing the period again, the effect diminishes, and after 140τ , $\Delta\delta$ is negligible (These are omitted from the graph for readability reasons and displayed in the appendix of this chapter, see Figure 6.10). This leads to the assumption that there is an optimum period and amplitude for the brush system studied. Especially in the lower periods studied, there is steep increase in the penetration depth when increasing the amplitude, for example for $P = 80$ this occurs when increasing A_z from 4 to 5 σ . This increase can be explained as follows: in order to accommodate larger particles, the polymer brush systems requires a certain minimum amount of perturbation. If the period is below a certain value, in this system 110τ , a sharp increase in $\Delta\delta$ is observed. For higher periods, this threshold is not reached, resulting in a gradual increase in penetration depth only due to the increase in amplitude. Interestingly, the amplitude at which the sharp transition occurs is at higher amplitudes for lower periods. This leads to the conclusion that there is an optimum period-amplitude relationship such that the brush oscillates in a synchronized way with the wall-oscillation, strengthened by the fact that there is such a relation for the brush-resonance in Figure 6.4. To further clarify this, the $\Delta\delta$ is plotted as function of size b and period P , in Figure 6.9. In this figure, the titles of the graph correspond to the amplitude A_z .

Figure 6.9: Penetration depth increase ($\Delta\delta$), normalized to the penetration depth at amplitude $A_z = 0 \sigma$, as function of size b , ranging from 1.8σ to 2.7σ and period, ranging from 70τ to 200τ . The individual graphs are labeled according to the amplitude applied, ranging from 0σ to 6σ .

From Figure 6.9 can be seen that the period and amplitude are closely related in terms of the penetration depth increase $\Delta\delta$. At $A_z = 3$, the optimal period is 100τ ; at $A_z = 4$, the optimal period is 90τ ; and so on. These values correspond exactly with the optimum frequencies and amplitudes in Figure 6.4. Therefore, it can be concluded that if one wants to optimize the penetration of nanoparticles in a polymer brush system, this system should be driven at its optimum frequency and amplitude. From $A_z = 6$ to $A_z = 10$, no significant change is observed from $A_z = 6$, hence these are omitted for clarity (see Figure 6.11).

In these simulations, an implicit solvent was used and all interactions were set to be repulsive. These assumptions are valid for systems where polymer-solvent and particle-solvent interactions are favored. When either polymer-polymer or polymer-particle interactions are varied, different results could be expected due to collapse of the brush,^[38] or particle-aggregation.^[37] Furthermore, the polymers used in this work are monodisperse, while synthesized polymers always entice a certain polydispersity,^[58] resulting in a broadening of b^* and $b_{\max}$ and the brush density profile.

The polymer brushes in this system are not (over)damped, therefore resonance behavior could be observed. For experimental brushes, the damping factor cannot be directly

determined, hence, further research is required to determine if this behavior can also be observed in experimental polymer systems. To provide more insight in this resonance behavior, MD simulations will be set up to determine the effect of the change in both grafting density and degree of polymerization on the resonance. Furthermore, the effect of changing the effective osmotic pressure of the particles (e.g. by changing the size of the simulation box) will be investigated.

6.4 Conclusions

In conclusion, MD simulations were performed on a polymer brush-particle system with an oscillating wall. By changing the amplitude and period of the oscillation the penetration depth of the particles could be increased up to 128%. Further research will be done on different types of oscillation as well as two-wall systems to study particle absorption in polymer-brush covered, oscillating microchannels such as the small intestine. Moreover, the effect of the degree of polymerization and grafting density on the optimal shaking conditions will be investigated.

6.5 References

- [1] S. T. Milner, *Science* **1991**, 251 (4996), 905 | 10.1126/science.251.4996.905
- [2] R. Barbey, L. Lavanant, D. Paripovic, N. Schuwer, C. Sugnaux, S. Tugulu, H. A. Klok, *Chem. Rev.* **2009**, 109 (11), 5437-527 | 10.1021/cr900045a
- [3] I. Banerjee, R. C. Pangule, R. S. Kane, *Adv. Mater.* **2011**, 23 (6), 690-718 | 10.1002/adma.201001215
- [4] M. Klein Gunnewiek, A. Di Luca, X. Sui, C. A. van Blitterswijk, L. Moroni, G. J. Vancso, *Isr. J. Chem.* **2012**, 52 (3-4), 339-346 | 10.1002/ijch.201100118
- [5] M. Klein Gunnewiek, E. M. Benetti, A. Di Luca, C. A. van Blitterswijk, L. Moroni, G. J. Vancso, *Langmuir* **2013**, 29 (45), 13843-52 | 10.1021/la403360r
- [6] M. E. V. Johansson, D. Ambort, T. Pelaseyed, A. Schütte, J. K. Gustafsson, A. Ermund, D. B. Subramani, J. M. Holmén-Larsson, K. A. Thomsson, J. H. Bergström, S. Van Der Post, A. M. Rodriguez-Piñeiro, H. Sjövall, M. Bäckström, G. C. Hansson, *Cell. Mol. Life Sci.* **2011**, 68, 3635-3641 | 10.1007/s00018-011-0822-3
- [7] J. E. G. Lipson, D. S. Gaunt, M. K. Wilkinson, S. G. Whittington, *Macromolecules* **1987**, 20 (1), 186-190 | 10.1021/ma00167a032
- [8] D. Reith, A. Milchev, P. Virnau, K. Binder, *Macromolecules* **2012**, 45 (10), 4381-4393 | 10.1021/ma202745b
- [9] M. Murat, G. S. Grest, *Phys. Rev. Lett.* **1989**, 63, 1074 | 10.1103/PhysRevLett.63.1074
- [10] K. Binder, T. Kreer, A. Milchev, *Soft Matter* **2011**, 7 (16), 7159 | 10.1039/c1sm05212h
- [11] S. A. Egorov, H. P. Hsu, A. Milchev, K. Binder, *Soft Matter* **2015**, 11 (13), 2604-16 | 10.1039/c4sm02862g
- [12] F. Costantini, E. M. Benetti, D. N. Reinhoudt, J. Huskens, G. J. Vancso, W. Verboom, *Lab on a chip* **2010**, 10, 3407-12 | 10.1039/c0lc00187b
- [13] I. Tokareva, S. Minko, J. H. Fendler, E. Hutter, *J. Am. Chem. Soc.* **2004**, 126 (49), 15950-1 | 10.1021/ja044575y

- [14] G. Subramanian, D. R. M. Williams, P. A. Pincus, *Macromolecules* **1996**, 29 (11), 4045-4050 | 10.1021/Ma946439r
- [15] J. U. Kim, B. O'Shaughnessy, *Macromolecules* **2006**, 39 (1), 413-425 | 10.1021/ma050817i
- [16] J. U. Kim, M. W. Matsen, *Macromolecules* **2008**, 41 (1), 246-252 | 10.1021/ma071906t
- [17] W. M. de Vos, F. A. M. Leermakers, A. de Keizer, J. M. Kleijn, M. A. Cohen Stuart, *Macromolecules* **2009**, 42 (15), 5881-5891 | 10.1021/ma900819b
- [18] L. M. Hall, K. S. Schweizer, *Soft Matter* **2010**, 6 (5), 1015 | 10.1039/b919160g
- [19] A. Halperin, M. Kröger, *Macromolecules* **2011**, 44 (17), 6986-7005 | 10.1021/ma201006h
- [20] A. Halperin, M. Kröger, E. B. Zhulina, *Macromolecules* **2011**, 44 (9), 3622-3638 | 10.1021/ma200068d
- [21] K. H. A. Lau, C. Ren, S. H. Park, I. Szleifer, P. B. Messersmith, *Langmuir* **2012**, 28 (4), 2288-2298 | 10.1021/la203905g
- [22] S. A. Egorov, *J. Chem. Phys.* **2012**, 137 (May), | 10.1063/1.4757017
- [23] A. Milchev, D. I. Dimitrov, K. Binder, *Polymer* **2008**, 49 (17), 3611-3618 | 10.1016/j.polymer.2008.04.032
- [24] V. Ermilov, A. Lazutin, A. Halperin, *Macromolecules* **2010**, 43 (7), 3511-3520 | 10.1021/ma9027273
- [25] H. Merlitz, C.-X. Wu, J.-U. Sommer, *Macromolecules* **2012**, 45 (20), 8494-8501 | 10.1021/ma301781b
- [26] T. Curk, F. J. Martinez-Veracoechea, D. Frenkel, J. Dobnikar, *Soft Matter* **2013**, 9 (23), 5565 | 10.1039/c3sm50486g
- [27] M. G. Opferman, R. D. Coalson, D. Jasnow, A. Zilman, *Langmuir* **2013**, 29 (27), 8584-8591 | 10.1021/la4013922
- [28] T. Curk, F. J. Martinez-Veracoechea, D. Frenkel, J. Dobnikar, *Nano Lett.* **2014**, 14 (5), 2617-2622 | 10.1021/nl500449x
- [29] J. Cheng, A. Vishnyakov, A. V. Neimark, *The Journal of Chemical Physics* **2015**, 142, 34705 | 10.1063/1.4905894
- [30] R. A. Gage, E. P. K. Currie, M. A. Cohen Stuart, *Macromolecules* **2001**, 34 (15), 5078-5080 | 10.1021/ma0018695
- [31] S. Santer, J. Rühle, *Polymer* **2004**, 45 (25), 8279 - 8297 | 10.1016/j.polymer.2004.09.085
- [32] E. Filippidi, V. Michailidou, B. Loppinet, J. Rühle, G. Fytas, *Langmuir* **2007**, 23 (13), 5139-5142 | 10.1021/la0637162
- [33] I. Luzinov, S. Minko, V. V. Tsukruk, *Soft Matter* **2008**, 4, 714-725 | 10.1039/B718999K
- [34] R. Oren, Z. Liang, J. S. Barnard, S. C. Warren, U. Wiesner, W. T. S. Huck, *J. Am. Chem. Soc.* **2009**, 131 (5), 1670-1671 | 10.1021/ja8090092
- [35] J. Yaneva, D. I. Dimitrov, A. Milchev, K. Binder, *J. Colloid Interface Sci.* **2009**, 336 (1), 51-8 | 10.1016/j.jcis.2009.03.062
- [36] J. U. Kim, B. O'Shaughnessy, *Phys. Rev. Lett.* **2002**, 89, 238301 | 10.1103/PhysRevLett.89.238301
- [37] O. A. Guskova, S. Pal, C. Seidel, *EPL (Europhysics Letters)* **2009**, 88 (3), 38006 | 10.1209/0295-5075/88/38006
- [38] Z. Lian, S. Qi, J. Zhou, F. Schmid, *J. Phys. Chem. B* **2015**, 119 (10), 4099-108 | 10.1021/jp511911g
- [39] D. Zhang, Y. Jin, J. Cheng, Y. Jiang, L. He, L. Zhang, *J. Polym. Sci., Part B: Polym. Phys.* **2014**, 52 (4), 299-309 | 10.1002/polb.23415

- [40] D. Zhang, J. Cheng, Y. Jiang, X. Wen, L. Zhang, *J. Polym. Sci., Part B: Polym. Phys.* **2013**, *51* (6), 392-402 | 10.1002/polb.23224
- [41] R. Oren, Z. Liang, J. S. Barnard, S. C. Warren, U. Wiesner, W. T. Huck, *J. Am. Chem. Soc.* **2009**, *131* (5), 1670-1 | 10.1021/ja8090092
- [42] S. Christau, T. Moller, Z. Yenice, J. Genzer, R. von Klitzing, *Langmuir* **2014**, *30* (43), 13033-41 | 10.1021/la503432x
- [43] M. Retsch, A. Walther, K. Loos, A. H. Muller, *Langmuir* **2008**, *24* (17), 9421-9 | 10.1021/la8009767
- [44] A. Milchev, D. I. Dimitrov, K. Binder, *Biomicrofluidics* **2010**, *4*, 32202 | 10.1063/1.3396446
- [45] L. Spirin, A. Galuschko, T. Kreer, K. Binder, J. Baschnagel, *Phys. Rev. Lett.* **2011**, *106*, 168301 | 10.1103/PhysRevLett.106.168301
- [46] L. Spirin, A. Galuschko, T. Kreer, *Macromolecules* **2011**, *44* (23), 9399 | 10.1021/ma2014029
- [47] I. V. Neratova, T. Kreer, J.-U. Sommer, *Macromolecules* **2015**, *48* (11), 3756-3766 | 10.1021/acs.macromol.5b00042
- [48] H. S. Li, B. K. Zhang, J. Li, W. D. Tian, K. Chen, *J. Chem. Phys.* **2015**, *143* (22), 224903 | 10.1063/1.4936921
- [49] G. S. Grest, K. Kremer, *Phys. Rev. A* **1986**, *33* (5), 3628 | 10.1103/PhysRevA.33.3628
- [50] S. de Beer, M. H. Müser, *Macromolecules* **2014**, *47* (21), 7666-7673 | 10.1021/ma501718b
- [51] S. Plimpton, *J. Comp. Phys.* **1995**, *117* (1), 1 | 10.1006/jcph.1995.1039
- [52] J. E. Jones, *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences* **1924**, *106* (738), 463-477 | 10.1098/rspa.1924.0082
- [53] J. D. Weeks, D. Chandler, H. C. Andersen, *The Journal of Chemical Physics* **1971**, *54* (12), 5237 | 10.1063/1.1674820
- [54] R. D. Groot, P. B. Warren, *J. Chem. Phys.* **1997**, *107* (11), 4423-4435 | Doi 10.1063/1.474784
- [55] C. Pastorino, T. Kreer, M. Müller, K. Binder, *Phys. Rev. E* **2007**, *76*, 26706 | 10.1103/PhysRevE.76.026706
- [56] W. Humphrey, A. Dalke, K. Schulten, *J. Molec. Graphics* **1996**, *14* (1), 33 | 10.1016/0263-7855(96)00018-5
- [57] M. Murat, G. S. Grest, *Macromolecules* **1989**, *22* (10), 4054-4059 | 10.1021/ma00200a041
- [58] C. Kang, R. M. Crockett, N. D. Spencer, *Macromolecules* **2014**, *47* (1), 269-275 | 10.1021/ma401951w

Appendix

Figure 6.10: Increase in penetration depth, normalized by the penetration depth at $A_z = 0 \sigma$, for particles of size 1.8 to 2.7σ , at $P = 150$ to 200τ .

Figure 6.11: Increase in penetration depth, normalized by the penetration depth at $A_z = 0 \sigma$, for particles of size 1.8 to 2.7σ , at $A_z = 7$ to 10σ .

Outlook

In this thesis, electrochemical experiments have been performed on particles in microchannels coated with a redox-responsive polymer. Furthermore, molecular dynamics simulations were done on particles in contact with a polymer-brush coated substrate. In future research the following directions could be explored. MD simulations of polymer brushes of various length and grafting densities could provide more insight in the resonance behavior observed in this thesis. Other directions could be the study of adsorption and desorption of nanoparticles in brush-covered microchannels with both static and dynamic brushes, and the application of various other polymer PFS systems in microfluidic devices. In case of the former, some preliminary results are shown below, realized with the aid of M.H.M. Willems, L. Pelin, G. Lajoinie, L. dos Ramos and S. de Beer.

7.1 Sorption of particles on PNIPAM covered microchannels

Since the surface-initiated polymerization of poly(*N*-isopropylacrylamide) (PNIPAM) is well studied,^[1-2] this polymer is used as model for a study on the adsorption and desorption of particles in polymer-coated microchannels. PNIPAM is tethered to the glass microchannel wall via surface-initiated atom-transfer radical polymerization (SI-ATRP). Subsequently, to verify that the polymer coating is indeed on the channel wall, two microfluidic chips were broken and imaged with a scanning electron microscope (SEM), one without polymer and one with polymer.

Figure 7.1: Cross-sectional SEM images of a microchannel: (a) without PNIPAM and (b) with PNIPAM tethered to the wall.

Figure 7.1 shows two SEM images of a cross-section of a microchannel. In the top image (a), no PNIPAM is attached to the channel wall. In the bottom image (b) a layer of PNIPAM is visible. Due to the confinement there is more polymer in the corners of the channel than in the flat top and bottom of the channel.

The presence of PNIPAM was also confirmed with contact angle measurements in the microchannel. The bare glass wall has a contact angle with water of 0° , which means it is perfectly wetting. In experiments following the deposition of the initiator, the contact angle increased to 63° . After the polymerization was performed, the contact angle was again 0° , indicating the polymerization was successful.

For the sorption experiments, Nile red was chosen as a fluorescent dye. To ensure a proper blank measurement could be made, a second set of microfluidic chips was functionalized with (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane by vapor deposition. These exhibit a high contact angle of 94° , resulting in a low adsorption of Nile red on the channel wall. The Nile red solution was pumped into the chips at $5 \mu\text{L min}^{-1}$, and every 5 seconds a fluorescent image was captured. The resulting adsorption and desorption graphs are displayed in Figure 7.2.

Figure 7.2: Fluorescent signals of (a) adsorption and (b) desorption experiments. Flow rate used was $5 \mu\text{L min}^{-1}$. The fluorinated microchannel (red dots) exhibits faster adsorption as well as desorption when compared to the PNIPAM-coated microchannel (blue dots).

In Figure 7.2, the height of the fluorescent signal is obtained by taking the mean of the red channel of the image. This has maximum value of 255, and minimum value of 0. In Figure 7.2a, the adsorption within the channel is displayed, while in Figure 7.2b, the desorption of Nile red from the channel is shown. From both figures can be concluded that the PNIPAM-functionalized channel shows both a slower increase as a slower decrease in fluorescent signal, indicating that the PNIPAM coating has a beneficial effect on particle retention. However, further study is required to make any well-founded conclusions on this system.

7.2 Towards redox-responsive brushes in microchannels

The PFS used in the electrochemical experiments in this thesis is a linear polymer, grafted to the gold electrodes. PFS is a versatile polymer that could be employed in a plethora of PFS-functionalized microfluidic devices in various forms. PFS hydrogels can be used for metal nanoparticle formation,^[3-4] and micrometer thick PFS hydrogels can be spin-coated on flat substrates.^[5] These could be directly applied to microfluidic devices using a filling/rinsing technique and subsequently used as in-channel metal nanoparticle foundries or electrocatalysts.^[5]

Recent work done by Elbert et al.^[6], showed that PFS can be grafted from a polystyrene nanoparticle by surface-initiated anionic ring-opening polymerization. As surface initiator, diphenylhexyllithium was used. This initiator was chosen because it does not initiate the polymerization of in-solution PFS-monomer, resulting in surface attachment of the polymer. To immobilize the initiator, the molecule reacted with the unreacted vinyl groups of the polystyrene nanoparticle. This surface-attachment procedure could be fairly straightforward transferred to a vinyl-covered gold surface, for example via an oct-7-ene-1-thiol monolayer.

7.3 Experimental section

Reagents and solvents: All chemicals were purchased from Sigma-Aldrich and used as received, except where noted otherwise. 3-(chlorodimethylsilyl)propyl 2-bromo-2-methylpropionate (ATRP initiator) was synthesized according to literature procedures.^[7] Toluene was purchased from Biosolve. Ethanol was purchased from Merck.

Fabrication of the microfluidic device: For these experiments the same microfluidic device was used as described in Chapter 4 and the appendix, except for two changes: (1) No electrodes were added and (2) the bonding process was done at 650°C for 1 hour.

Microchannel functionalization: The microfluidic chips were cleaned with O₂-plasma for 2 hours. Subsequently, the ATRP initiator was deposited in the microchip by vapor deposition under vacuum. Afterwards, the chip was flushed with toluene and ethanol. 20 mmol *N*-isopropylacrylamide (NIPAM) and 0.4 mmol *N,N,N',N',N'*-pentamethyldiethylenetriamine (PMDETA) were dissolved in a water-methanol mixture

(8 mL, 1:9 v/v). Cu(I)Br (0.2 mmol) and Cu(II)Br₂ (0.02 mmol) were added and left to equilibrate for 30 minutes. The reaction mixture was inserted into the microfluidic device via a syringe pump and left to react for 10 minutes. Afterwards the chip was rinsed with water for 30 minutes and ethylenediaminetetraacetic acid (EDTA, 0.1M) for 5 minutes and dried in a nitrogen stream. The whole reaction was performed in an oxygen-free atmosphere.

Contact angle measurements: Contact angle images were captured with an Olympus DP71 camera, by using an Olympus IX71 microscope at 100x magnification under ambient conditions and subsequently analyzed using SCA20 software.

Fluorescence measurements: Nile red (NR, 5 μ M) was dissolved in ethanol and used as fluorescent marker and measured via an Olympus IX71 optical microscope. For the adsorption experiments an ethanol-filled channel was filled with the dye containing solution at 5 μ L min⁻¹. For the desorption experiments the same channel was used, which was still filled with the dye containing solution and subsequently filled with ethanol at 5 μ L min⁻¹. During the experiments, every 5 seconds an image was captured.

7.4 References

- [1] K. Matyjaszewski, J. Xia, *Chem. Rev.* **2001**, 101 (9), 2921-2990 | 10.1021/cr940534g
- [2] H.-i. Lee, J. Pietrasik, S. S. Sheiko, K. Matyjaszewski, *Prog. Polym. Sci.* **2010**, 35 (1-2), 24-44 | 10.1016/j.progpolymsci.2009.11.002
- [3] X. Sui, X. Feng, M. A. Hempenius, G. J. Vancso, *J. Mater. Chem. B* **2013**, 1, 1658 | 10.1039/C3TB00209H
- [4] B. Zoetebier, M. A. Hempenius, G. J. Vancso, *Chem. Commun.* **2014**, 1-4 | 10.1039/C4CC06988A
- [5] X. Feng, H. Wu, X. Sui, M. A. Hempenius, G. Julius Vancso, *Eur. Polym. J.* **2015**, 72, 535-542 | 10.1016/j.eurpolymj.2015.05.022
- [6] J. Elbert, H. Didzoleit, C. Fasel, E. Ionescu, R. Riedel, B. Stuhn, M. Gallei, *Macromol. Rapid Commun.* **2015**, 36 (7), 597-603 | 10.1002/marc.201400581
- [7] B. Lego, W. G. Skene, S. Giasson, *Langmuir* **2008**, 24 (2), 379-82 | 10.1021/la703051b

Stepwise fabrication of the microfluidic device

A.1 Mask 1

A.1.1 Mask design

The first mask used in the fabrication of the microfluidic device is shown below, and is used for the definition of the microfluidic channels.

Figure A.1: Mask used for channel definition.

A.1.2 Process flow

Step	Process	Process parameters
1	Cleaning HNO ₃ 99% glass	NL-CLR-WB16 <ul style="list-style-type: none"> • Beaker 1: HNO₃ (99%) 5 min • Beaker 2: HNO₃ (99%) 5 min
2	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 ΩM
3	Substrate drying	NL-CLR-WB Single wafer dryer <ul style="list-style-type: none"> • speed: 2500 rpm, 60 sec with 30 sec N₂ flow
4	Evaporation of Cr (Wafersfilm128)	NL-CLR- Balzers BAK600 <ul style="list-style-type: none"> • Crucible: 3 (Chromium) • Voltage: 8kV • Emission current: see mis logbook • Base pressure: < 10⁻⁶ mBar • Density: 7.2 • Depositionrate: 1 - 20 Å s⁻¹ • 10 nm
5	Evaporation of Au (Wafersfim144)	NL-CLR-Balzers BAK 600 <ul style="list-style-type: none"> • Crucible: 2 (Au) • Voltage: 10 kV • Emission Current: see mis logbook • Base pressure: < 10⁻⁶ mBar • Density 19.3 g cm⁻³ • Deposition rate 1-10 Å s⁻¹ MAX. THICKNESS: 500nm <ul style="list-style-type: none"> • 100 nm

6	Dehydration bake	NL-CLR-WB21/22 dehydration bake at hotplate • temp. 120°C • time: 5 min
7	Coating Olin Oir 907-17	NL-CLR-WB21 Coating: • olin oir 907-17 • spin Program: 4000 (4000rpm, 30sec)
8	Pre exposure bake	Pre exposure bake: hotplate • time 90 sec • temp 95°C
9	Alignment & Exposure Olin OiR 907-17	NL-CLR- EV620 Electronic Vision Group EV620 Mask Aligner • Hg-lamp: 12 mW cm ⁻² • Exposure Time: 8 sec
10	Development Olin OiR resist	NL-CLR-WB21 After exposure Bake : hotplate • time 60 sec • temp 120°C development: developer: OPD4262 • time: 30 sec in beaker 1 • time: 30 sec in beaker 2
11	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 ΩM
12	Substrate drying	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N ₂ flow
13	Postbake Olin OiR resist	NL-CLR-WB21

		postbake: Hotplate • temp 120°C • time 15 min
14	Etching of gold (Wafersetch136)	NL-CLR-WB06 Use dedecated beaker with gold etch • recipe: $K_1:I_2:DI = (4:1:40)$ • add 40 g K_1 and 10 g I_2 to 400 ml DI water • temp.: 20°C, 30 seconds Etchrates = 200 nm min ⁻¹ (check rate with dummy wafer!) Excessive underetching of Cr occurs because of a galvanic reaction with gold. To minimize this make sure you do not overetch the chromium.
15	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 ΩM
16	Etching of chromium (Wafersetch134)	NL-CLR-WB6 Use dedicated beaker with chromium etch (standard) • temp.:20°C, 5 seconds Etchrates = 60 nm min ⁻¹ , Check always the etchrate with dummy wafer!
17	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 ΩM

18	Substrate drying	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N ₂ flow
19	Surface profile measurement (#metro105)	NL-CLR-Veeco Dektak 8 Measure thickness of Olin-layer + Au/Cr film prior to etching (note in logbook!)
20	Etching in HF 25%	NL-CLR-WB09 or 10 Use private beaker for etching: HF (25%) Add HF (50%) to DI for diluted etch solutions (Add HF to water!) • temp.: 20°C Etch rates (function of load): • Borofloat BF33: 1 $\mu\text{m min}^{-1}$ (5 μm , 5 minutes) • MEMPax: 1 $\mu\text{m min}^{-1}$ (5 μm , 5 minutes)
21	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 ΩM
22	Substrate drying	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N ₂ flow
23	Stripping resist with Acetone	NL-CLR-WB-11 Rinse with Acetone VLSI
24	Stripping resist in HNO ₃ (99%) (#lith194)	NL-CLR-WB-16 • beaker 0: HNO ₃ (99%) • time: > 10 min, depends on the application
25	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec

		3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is $> 10 \Omega\text{M}$
26	Substrate drying	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N_2 flow
27	Surface profile measurement (#metro105)	NL-CLR-Veeco Dektak 8 Measure thickness of Olin-layer + Au/Cr film post to HF-etching (note in logbook!)
28	Etching of gold (Wafersetch136)	NL-CLR-WB06 Use dedecated beaker with gold etch • recipe: $\text{K}_1:\text{I}_2:\text{DI} = (4:1:40)$ • add 40 g K_1 and 10 g I_2 to 400 ml DI water • temp.: 20°C , 120 s Etchrates = 200 nm min^{-1} (check rate with dummy wafer!) Excessive underetching of Cr occurs because of a galvanic reaction with gold. To minimize this make sure you do not overetch the chromium.
29	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is $> 10 \Omega\text{M}$
30	Etching of chromium (Wafersetch134)	NL-CLR-WB6 Use dedicated beaker with chromium etch (standard) • temp.: 20°C , 30 s Etchrates = 60 nm min^{-1} , Check always the etchrate with dummy wafer!
31	Quick Dump Rinse (QDR)	NL-CLR-Wet benches

		<p>Recipe 1 QDR: 2 cycles of steps 1 till 3,</p> <p>1- fill bath 5 sec</p> <p>2- spray dump 15 sec</p> <p>3- spray-fill 90 sec</p> <p>4- end fill 200 sec</p> <p>Recipe 2 cascade rinsing: continuous flow</p> <p>Rinse till the DI resistivity is $> 10 \Omega\text{M}$</p>
32	Substrate drying	<p>NL-CLR-WB</p> <p>Single wafer dryer</p> <ul style="list-style-type: none"> • speed: 2500 rpm, 60 sec with 30 sec N_2 flow
33	Surface profile measurement (#metro105)	<p>NL-CLR-Veeco Dektak 8</p> <p>Measure thickness of Olin-layer + Au/Cr film post to HF-etching</p> <p>(note in logbook!)</p>
34	Inspection by optical microscope	<p>NL-CLR- 6 microscopes</p> <ul style="list-style-type: none"> • Olympus Microscope (4) • Leica Microscope (2) <p>Use Olympus BH-2 with Analysis software, magn. 5x, 20x and 50x.</p> <p>Save files in tiff-format</p>

A.2 Mask 2

A.2.1 Mask design

The mask used for the embedded electrodes is shown below.

Figure A.2: The mask used for the embedded electrodes.

A.2.2 Process flow

Step	Process	Process parameters
1	Cleaning HNO ₃ 99% glass	NL-CLR-WB16 • Beaker 1: HNO ₃ (99%) 5 min • Beaker 2: HNO ₃ (99%) 5 min
2	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec

		3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 Ω M
3	Substrate drying	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N ₂ flow
4	Dehydration bake	NL-CLR-WB21/22 dehydration bake at hotplate • temp. 120°C • time: 5 min
5	Coating Olin Oir 907-17	NL-CLR-WB21 Coating: • olin oir 907-17 • spin Program: 4000 (4000 rpm, 30 sec)
6	Pre exposure bake	Pre exposure bake: hotplate • time 90 sec • temp 95°C
7	Alignment & Exposure Olin OiR 907-17	NL-CLR- EV620 Electronic Vision Group EV620 Mask Aligner • Hg-lamp: 12 mW cm ⁻² • Exposure Time: 30 sec
8	Development Olin OiR resist	NL-CLR-WB21 After exposure Bake : hotplate • time 60 sec • temp 120°C development: developer: OPD4262 • time: 30 sec in beaker 1 • time: 30 sec in beaker 2
9	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec

		4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 Ω M
10	Substrate drying	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N ₂ flow
11	Postbake Olin OiR resist	NL-CLR-WB21 postbake: Hotplate • temp 120°C • time 15 min
12	Etching SiO ₂ BHF (1:7) (#etch125)	NL-CLR-WB9 or 10 Use private beaker with BHF (1:7) • temp.: 20°C, time: 5 min Etchrates: • thermal SiO ₂ : 60-80 nm min ⁻¹ • PECVD SiO ₂ : 125 nm min ⁻¹ • TEOS SiO ₂ : 180 nm min ⁻¹ • TEOS H3 (new) : 242 nm min ⁻¹ • Pyrex #7740: 20 nm min ⁻¹ • Borofloat BF33: 20-25 nm min ⁻¹ • Si ₃ N ₄ -H ₂ : 0.64 nm min ⁻¹
13	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 Ω M
14	Substrate drying	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N ₂ flow
15	Evaporation of Cr (Wafersfilm128)	NL-CLR- Balzers BAK600 • Crucible: 3 (Chromium)

		<ul style="list-style-type: none"> • Voltage: 8kV • Emission current: see mis logbook • Base pressure: $< 10^{-6}$ mBar • Density: 7.2 • Depositionrate: $1 - 20 \text{ \AA s}^{-1}$ • 10 nm
16	Evaporation of Au (Wafersfim144)	NL-CLR-Balzers BAK 600 <ul style="list-style-type: none"> • Crucible: 2 (Au) • Voltage: 10 kV • Emission Current: see mis logbook • Base pressure: $< 10^{-6}$ mBar • Density 19.3 g cm^{-3} • Deposition rate $1-10 \text{ \AA s}^{-1}$ MAX. THICKNESS: 500nm <ul style="list-style-type: none"> • 100 nm
17	Lithography - Lift-off procedure for metals (WafersLith144)	NL-CLR- Wet-Bench 11 Use ultrasonic bath Use metal beaker and waferholder <ul style="list-style-type: none"> • Beaker 1: Aceton technical, > 10 min • Beaker 2: Aceton VLSI , >10 min • Beaker 3: Isopropanol VLSI > 10 min
18	Quick Dump Rinse (QDR)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 5 sec 2- spray dump 15 sec 3- spray-fill 90 sec 4- end fill 200 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is $> 10 \text{ \Omega M}$
19	Substrate drying	NL-CLR-WB Single wafer dryer <ul style="list-style-type: none"> • speed: 2500 rpm, 60 sec with 30 sec N_2 flow

A.3 Mask 3

A.3.1 Mask design

The mask used for the powderblasted holes is shown below.

Figure A.3: Mask used for the powderblasting.

A.3.2 Process flow

Step	Process	Process parameters
1	Clean HNO ₃ 1&2 (#clean105)	NL-CLR-WB16 • Beaker 1: HNO ₃ (99%) 5 min • Beaker 2: HNO ₃ (99%) 5 min
2	Quick Dump Rinse (QDR) (#clean119)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 15 sec 2- spray dump 15 sec 3- spray-fill 40 sec 4- end fill 500 sec

		Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is $> 10 \Omega\text{M}$
3	Substrate drying (#clean120)	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 60 sec with 30 sec N_2 flow
4	Laminate PB foil	NL-CLR-EVG 20 Set laminator to 105°C Cut foil, place back in refrigerator Place wafer on CR paper Remove protection layer 1 of foil User roller to place foil bubble free on wafer Put CR paper on top Feed to laminator Cut the wafer free after lamination
5	Alignment and exposure (#lith135)	NL-CLR-EVG 20 Electronic Vision Group 20 Mask Aligner • Hg-lamp: 12 W cm^{-2} • Exposure time: 15 sec
6	Removal of protecting layer	NL-CLR-WB21 Place wafer on 95°C heating plate for a few seconds Remove protecting layer 2
7	Back protection	NL-CLR-Back end Apply dicing foil to other side of wafer Heat wafer at 70°C for ~ 1 minute
8	Development	NL-Carre-BIOS-Floor 2 Use an aqueous 0.2% soda solution, 35°C Develop wafer for ~ 10 minutes
9	Powderblasting of glass (#etch120)	NL-Carre-BIOS Powderblaster For feature size $> 100 \mu\text{m}$ • Particles: $29 \mu\text{m Al}_2\text{O}_3$ • Pressure: 5 bar
10	Removal of foil and particles after powderblasting (#clean139)	Outside cleanroom - use own facility Start with removal of foil • remove dicing foil manually • remove powderblast foil manually • rinse wafer with water (by spraying) to remove powderblast particles • strip foil in Na_2CO_3 solution, time > 30 min

		<ul style="list-style-type: none"> • rinse wafer with water time > few minutes • ultrasonic cleaning in water, time >10 min • ultrasonic cleaning in fresh water, time >10 min • drying of substrate by spinning or N₂ gun
11	Removal of particles (#clean110)	NL-CLR-Wet Bench11 Removal of particles generated by powderblasting and/or metal lift-off. Use ultrasonic bath 1 Use dedicated metal beakers and carriers <ul style="list-style-type: none"> • beaker 1: Aceton technical, > 10 min , ultrasonic • beaker 2: Isopropanol technical > 10min, ultrasonic • beaker 3: DI water > 10min, ultrasonic
12	Quick Dump Rinse (QDR) (#clean119)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 15 sec 2- spray dump 15 sec 3- spray-fill 40 sec 4- end fill 500 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 ΩM
13	Substrate drying (#clean120)	NL-CLR-WB Single wafer dryer <ul style="list-style-type: none"> • speed: 2500 rpm, 60 sec with 30 sec N₂ flow

A.4 Bonding and dicing procedure

A.4.1 Process flow

Step	Process	Process parameters
1	Cleaning HNO ₃ 99% glass	NL-CLR-WB16 <ul style="list-style-type: none"> • Beaker 1: HNO₃ (99%) 5 min • Beaker 2: HNO₃ (99%) 5 min
2	Quick Dump Rinse (QDR) (#clean119)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 15 sec 2- spray dump 15 sec 3- spray-fill 40 sec 4- end fill 500 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is > 10 ΩM
3	Cleaning in Piranha (#clean117)	NL-CLR-WB09 Applications: <ul style="list-style-type: none"> • For direct wafer bonding use dedicated beaker and always a fresh solution. • For polymer stripping it is allowed to use "aged" solutions and can be re-used by adding H₂O₂ if necessary. Mixture: H ₂ SO ₄ :H ₂ O ₂ (3:1) vol% <ul style="list-style-type: none"> • fill beaker with H₂SO₄ • add slowly H₂O₂ and avoid a temperature rise above 100°C • Set stirrer to 20% • start cleaning (load wafers) when solution temperature is between 85-90°C • cleaning time wafers with electrodes 1 min • cleaning time 15 min
4	Quick Dump Rinse (QDR) (#clean119)	NL-CLR-Wet benches Recipe 1 QDR: 2 cycles of steps 1 till 3, 1- fill bath 15 sec 2- spray dump 15 sec 3- spray-fill 40 sec

		4- end fill 500 sec Recipe 2 cascade rinsing: continuous flow Rinse till the DI resistivity is $> 10 \Omega\text{M}$
5	Substrate drying (#clean120)	NL-CLR-WB Single wafer dryer • speed: 2500 rpm, 150 sec with 75 sec N ₂ flow
6	Manually Aligning & Prebonding (#bond101)	NL-CLR-WB16 • Contact wafers manually • Apply light pressure with tweezers • If necessary use tweezers to press out air-bubbles • Check prebonding by using IR-setup
7	Wafer bonding glass-glass (#therm132)	NL-CLR-Furnace E2 • Temp.: 550 °C • Gas: N ₂ • Time: 1 hour
8	Dicing foil Nitto SWT 10 (#back103)	NL-CLR dicing room Nitto SWT 10 dicing foil
9	Dicing of a glass wafer (#back102)	NL-CLR-Dicing room Use Load point Micro Ace 3 Parameters dicing: Wafer work size: 110 mm for a standard 100 mm silicon wafer Feed speed: 5 mm s ⁻¹ Max cut depth: 1000 μm per cycle

The aim of this thesis has been to provide the ground work for the electrochemical sensing of analytes in polymer-functionalized microchannels via gold-coated microfabricated microchannel electrodes and for the in-situ study of the adsorption of particles in polymer brushes through molecular dynamics simulations. Both these objectives encompass polymer systems that are out of equilibrium and reveal how particles interact with these polymers. The aim was accomplished via the successful fabrication of in-channel gold-coated electrodes within a microfluidic device. To these gold-coated electrodes a redox-active polymer, poly(ferrocenylsilane) (PFS), was attached, via which sodium ascorbate could be sensed. To further understand the effect of particles in polymer systems, molecular dynamics (MD) simulations were performed on the insertion of particles of various size, shape and orientation, into a polymer brush. The force-distance curves were successfully modelled by two different models, one using scaling arguments and one aided through MD. Furthermore, the effect of shaking the wall, to which the polymer brushes are attached, on the penetration depth of particles into the brush was simulated, to approximate biological microsystems. From these could be concluded that if the brush is oscillated at its resonance frequency at a complementary resonance amplitude, the increase in penetration depth is at its maximum.

Chapter 1 consists of a short introduction into the topic of this work, as well as an overview of the thesis.

Chapter 2 encompasses a short review of the most relevant research done on the fabrication of in-channel electrodes, the in-chip electrochemical detection of various analytes and the MD simulations performed on polymer brushes. Furthermore, a concise overview of the electrochemical methods is given, and an introduction into the topic of MD is presented.

Chapter 3 covers the use of polymer coatings in glass microfluidic devices in depth, where polymers were employed both as active and passive layers.

Chapter 4 describes various electrochemical experiments performed on a microfluidic device with integrated gold electrodes. Both the fabrication and characterization of the device are discussed. In this device, a PFS layer was grafted to the gold electrodes, via which sodium ascorbate could be detected by electrochemical means.

Chapter 5 concerns the first of the two MD-focused chapters. This chapter describes how particles of various size, orientation and shape, were inserted into a polymer brush, resulting in different inclusion energies. The forces on the particles were modelled via two models, and a size- and shape-dependent pre-factor could be determined.

In chapter 6, the second MD simulation is reported, where a polymer brush is shaken at the grafting wall. The effect of this motion on the penetration depth of spherical particles is determined. By varying the size of the particles and the amplitude and period of oscillation, an optimum period and amplitude for particle absorption could be found. This optimum corresponded with a resonance period and complementary amplitude of oscillation.

Finally, chapter 7 gives an outlook towards future research on brush-covered microchannels. Moreover, recommendations for other applications of PFS within microchannels is provided.

Het doel van dit proefschrift was tweeledig. Het eerste bevatte het leggen van de fundamenteën voor de elektrochemische detectie van deeltjes in met polymeer beklede microkanalen door middel van aangebrachte gouden micro-elektroden. De tweede doelstelling omvatte het leggen van de fundamenteën voor het onderzoek naar de opname van deeltjes in polymeerborstels door middel van moleculaire dynamicasimulaties (MD-simulaties). Beide doelstellingen bevatten polymeersystemen die uit evenwicht zijn en onthullen hoe de deeltjes en de polymeer op elkaar reageren. De doelstellingen zijn behaald met behulp van de fabricage van gouden elektroden in microkanalen in een microfluïdisch apparaat. Op deze gouden micro-elektroden was een redox-actieve polymeer aangebracht, te weten poly(ferrocenylsilaan) (PFS), waarmee natriumascorbaat gedetecteerd kon worden. Om beter te kunnen begrijpen wat het effect is van deeltjes in polymeersystemen zijn er MD-simulaties uitgevoerd met deeltjes van verschillende grootte, vorm en oriëntatie die in polymeerborstels werden geplaatst. De metingen betreffende kracht en afstand die hieruit volgden zijn vervolgens met succes gemodelleerd met twee verschillende modellen. Daarna is via MD-simulaties onderzocht wat het effect is van het schudden van de wand met polymeerborstels op de absorptiediepte van sferische deeltjes. Dit kan gebruikt worden als een model voor biologische systemen. Uit deze simulaties volgde dat de toename in absorptiediepte het hoogst is als er geschud wordt met de resonantiefrequentie en corresponderende amplitude van de polymeerborstel.

In hoofdstuk 1 wordt een korte introductie gegeven over het onderwerp van dit onderzoek. Tevens wordt er een overzicht van het proefschrift gegeven.

Hoofdstuk 2 geeft kort weer wat het meest relevante onderzoek is met betrekking tot elektrodes in microkanalen, elektrochemische detectie van deeltjes in microfluïdische chips en MD-simulaties van polymeerborstels. Daarnaast zijn de gebruikte elektrochemische methoden beschreven en is er een korte introductie in MD-simulaties gegeven.

Hoofdstuk 3 gaat in detail in op verschillende polymeercoatings van microkanalen in microfluïdische apparaten. Zowel actieve als passieve lagen worden behandeld.

Hoofdstuk 4 beschrijft meerdere elektrochemische experimenten die zijn uitgevoerd op een microfluïdisch apparaat, dat geïntegreerde gouden elektroden bevatte. Zowel de fabricage als de karakterisering van het apparaat worden besproken. Er werd een laag PFS aangebracht op de gouden elektroden waarmee via elektrochemie natriumascorbaat gedetecteerd kon worden.

Hoofdstuk 5 betreft het eerste van de twee op MD-simulaties gerichte hoofdstukken. Er werden deeltjes van verschillende grootte, oriëntatie en vorm in een polymeerborstel geplaatst. Dit zorgde voor een verandering in de benodigde energie voor de opname van het deeltje. De krachten op de deeltjes zijn in model gebracht met twee verschillende modellen en daarmee kon een vormafhankelijke voorfactor bepaald worden.

In hoofdstuk 6 wordt de tweede MD-simulatie uiteengezet. Een polymeerborstel werd geschud door middel van het bewegen van de wand waar de borstel aan vast zat. Door het variëren van de grootte van de deeltjes, de periode en de amplitude van de oscillatie kon een optimale periode en amplitude gevonden worden voor de absorptie van deeltjes. Deze optimale instellingen waren gelijk aan de resonantiefrequentie en de hieraan gekoppelde amplitude van de oscillatie.

Tenslotte wordt in hoofdstuk 7 een vooruitblik gegeven naar toekomstig onderzoek naar microkanalen bedekt met polymeerborstels. Daarnaast worden aanbevelingen gegeven voor andere toepassingen van PFS-gefunctionaliseerde microkanalen.

During my PhD period, nearing 4.5 years of work, one can imagine there are a lot of others involved, to help, aide and guide me. This is an attempt to thank those involved.

The long list starts with my professor and promotor: Julius Vancso. Julius, you have been an inspiration throughout my PhD life. Every time I left your office after a meeting, whether we talked about what was missing or what was going well, I was remotivated to improve the way I worked and tackle challenges. Your constant guidance has been invaluable throughout the project.

My assistant promotor and daily supervisor: Peter, thank you for teaching me to be an independent researcher. When the chips were down, you were always there with moral support and scientific ideas.

Sissi, je hebt me geïntroduceerd in de mooie wereld van de MD simulaties en daarvoor ben ik je erg dankbaar. Ik had nooit gedacht zoveel plezier te vinden in het opzetten en analyseren van computersimulaties. Maar dat niet alleen, ook je positieve instelling, eerst als directe buurvrouw en later als begeleider, heeft me geïnspireerd tijdens mijn promotietraject.

Ook wil ik Joost bedanken. Allereerst vanwege de synergie die we hadden tijdens de lunchpauzes en andere MTP activiteiten. Ik heb me mede dankzij jou altijd vermaakt op de werkvloer. Ook heb je me laten zien hoe je een echte DPD beleeft. Later gingen we samenwerken op wetenschappelijk gebied en je was een echte katalysator voor mijn labwerk en daaruit voortvloeiende resultaten. Je hulp bleek onmisbaar voor dit proefschrift.

My paranympths, Lionel and Andreas. Lionel, thanks for all the fun times we had during conferences, dinners, group outings and borrels. We started this journey together on the same project, and we finish against all odds in the same month. You've been an excellent coworker and friend. Andreas, you joined our group a little later, but fitted in immediately. I still remember (most of) the group trip to Darmstadt and the fun we had. Your commitment for BVB never ceased to amaze me. In short, office 4237 was always a good place to be.

Clemens, bedankt voor al het harde werk in het lab, de wetenschappelijke tips, en voor alle gezelligheid tijdens het werk. Je was meestal moeilijk te vinden maar altijd in de buurt voor een praatje of potje tafelvoetbal. Geneviève bedankt voor de mooie omslag, prachtig werk. Ook je nuchtere houding tijdens het promotietraject heeft me veel goeds gedaan. De (andere) dames van het secretariaat, Lidy, Hanke, Marion, bedankt voor de koffiemomentjes en andere leuke gesprekken.

Mark bedankt voor alle chemische kennis, je stond altijd klaar om tips te geven en te zorgen dat ik mezelf niet te erg verwondde in het lab.

Michel, bedankt dat je me wel eens liet winnen met tafelvoetbal, ook je tips voor de AFM zijn zeer nuttig geweest. Bram, je kwam pas later in mijn kantoor te zitten, maar was een goede aanvulling op het geheel. Ook daarvoor heb ik met plezier met je samen gewerkt. Marco, craziness incarnate, we had excellent times with Oban when you were a student, later as colleague you were my best chocolate fixer. I wish you all the best. Shanqui, Yunlong, Kaihuan, thanks for the fun times and the work we did together, I hope you all finish your PhD soon successfully. Liz and Mathies, the work you did with and for me as students is an excellent addition to this thesis.

I'd like to thank the cleanroom staff for their almost boundless process-knowledge, but also for their patience and joyful work attitude. Edin and Kees, thanks for helping me in the cleanroom whenever I had questions or challenges.

Hairong, Xueling, Aysegul, Wilma, Edit and Xiaofeng, you left me with fine examples how to successfully finish a PhD and taught me quite some along the way. Eddy, your frequent visits were always fun and interesting. Hubert and Lucie, newly arrived post-docs, I wish you good luck with the Asylum AFM and the scientific results.

And I'd like to thank all the MTP students whom I had the pleasure to meet, occasionally guide and mainly distract with coffee breaks and odd conversation: Jin, Lars, Maria, Keshav, Alex, Yuanyuan, Thomas, Natasha, Karthiga, Kim, Hermen, Barbara, Jing, Alireza, Ludger, Nico, Tom, Ramon, Lidia, Henrieke, Tatjana, Simone, Rik, Edo, Anne, Sida, Chongnan and Luuk (I hope I mentioned everybody).

De aikidoka's van Aikikan Aikido en natuurlijk Siavaish bedankt voor alle mooie trainingsuurtjes, mijn hoofd was altijd weer helder na een training.

Rijco, Wouter, Menno, en al die andere vrienden die al mijn klagen en successen aanhoorden, bedankt! René I was honored to be your paranymp, I hope little Marcus doesn't keep you up at night.

Pa en Ma, jullie bedankt voor de steun als het moeilijk was en de tijd om naar mijn verhalen te luisteren als het weer goed ging. Erg bedankt voor het oppassen zodat ik kon schrijven en voor alle relaxweekendjes in het Veen.

Lieve Jenneke, bedankt voor alles. Een paar regels in dit proefschrift kunnen niet weergeven wat je allemaal voor me betekend hebt. Je bent de beste vrouw die ik had kunnen wensen. Lieve Mattias, je leest dit waarschijnlijk pas vele jaren later, maar je hebt me zonder het te weten al ondersteund en geholpen met je vrolijke zelf.

Tenslotte dank ik God voor het intellect en het doorzettingsvermogen waarmee ik dit promotietraject tot een succesvol einde hoop te brengen. Ik wil me op niets anders laten voorstaan dan het kruis van Jezus Christus, mijn Heer.

Bart

Bernard Dingeman Kieviet (Bart) was born on the 11th of April 1984 in Utrecht, the Netherlands. In 2009, he earned his Bachelor's degree in Applied Physics at the University of Twente, Enschede, the Netherlands, and in 2011 his Master's degree in Nanotechnology at the same university. In that year, he joined prof. G.J. Vancso's group Materials Science and Technology of Polymers as a PhD-candidate, and the work performed over the course of four years is described in this thesis.

List of publications

Adhesion forces in AFM of redox responsive polymer grafts: Effects of tip hydrophilicity

X. Feng, B.D. Kieviet, J. Song, P.M. Schön, G.J. Vancso.

Applied Surface Science **2014**, 292, 107-110 | 10.1016/j.apsusc.2013.11.087

Stimulus-responsive polymers and other functional polymer surfaces as components in glass microfluidic channels

B.D. Kieviet, P.M. Schön, G.J. Vancso.

Lab on a Chip **2014**, 14 (21), 4159-4170 | 10.1039/c4lc00784k

Cosolvency-induced switching of the adhesion between Poly(methyl methacrylate) brushes

Y. Yu, B.D. Kieviet, E. Kutnyanszky, G.J. Vancso., S. de Beer.

ACS Macro Letters **2015**, 4 (1), 75-79 | 10.1021/mz500775w

Stretching of Collapsed Polymers Causes an Enhanced Dissipative Response of PNIPAM Brushes near their LCST

Yu, Y., B.D. Kieviet, F. Liu, I. Siretanu, E. Kutnyánszky, G.J. Vancso, S. de Beer.

Soft Matter **2015**, 11, 8508-8516 | 10.1039/c5sm01426c

Electrochemical atomic force microscopy reveals potential stimulated height changes of redox responsive Cu-azurin on gold

H. Wu, X. Feng, B.D. Kieviet, K. Zhang, H.J.W. Zandvliet, P.M. Schön, G.J. Vancso.

European Polymer Journal **2015** | 10.1016/j.eurpolymj.2015.12.002

Geometry-dependent insertion-forces on particles in swollen polymer brushes

S. de Beer, L.I.S. Mensink, B.D. Kieviet.

Macromolecules **2016**, 49 (3), 1070-1078 | 10.1021/acs.macromol.5b01960

Employing Co-Nonsolvency of Poly(N-isopropyl acrylamide) Brushes to Tune Adhesion and Friction

Y. Yu, B.D. Kieviet, G.J. Vancso., S. de Beer.

2016, submitted

Redox Control of Capillary Filling Speed in Poly(ferrocenylsilane)-Modified Microfluidic Channels for Switchable Delay Valves

L. Dos Ramos, G. Lajoinie, B.D. Kieviet, S. de Beer, M. Versluis, M.A. Hempenius, G.J. Vancso.

2016, submitted

In-line sensing of Sodium Ascorbate using a poly(ferrocenylsilane)-coated microfluidic device

B.D. Kieviet, L. Dos Ramos, M.A. Hempenius, J. Duvigneau, P.M. Schön, G.J. Vancso.

2016, submitted

Publications in preparation

Optimal shaking conditions for particle-absorption in swollen polymer brushes

B.D. Kieviet, G.J. Vancso., S. de Beer.

Controlled cutting of PNIPAM brush chains at their roots by photocleaving to provide grafting density variations for adhesion hysteresis and friction studies

L. Dos Ramos, E.M. Benetti, B.D. Kieviet, H. Gojzewski, S. de Beer, M.A. Hempenius, G.J. Vancso.