# Vesicular Structures Self-Assembled from Oligonucleotide-Polymer Hybrids: Mechanical Prevention of Bacterial Colonization Upon their Surface Tethering Through Hybridization

*Nicolas Cottenye, Karine Anselme, Lydie Ploux, and Corinne Vebert-Nardin**

**In order to design soft coatings, surface tethering of vesicular structures self-assembled from oligonucleotide-polymer hybrids is achieved through hybridization. Watson-Crick base-pairing occurs between the nucleotide sequences involved in the self-assembly and their surface-tethered complementary sequences. Combining the quartz crystal microbalance and in situ observations using confocal laser scanning microscopy, it is evidenced that the vesicles retain their morphology even under flow stress. Surprisingly, these soft surfaces prevent bacterial colonization.**

## 1. Introduction

Owing to the plethora of potential applications in biosensing,[1,2] drug delivery as well as targeted immobilization,[3] the surface tethering of vesicular structures through Watson-Crick base pairing between complementary nucleotide sequences is a current dynamic field of research. For this purpose, single stranded nucleotide sequences are usually coupled to liposomes subsequent to their supramolecular assembly prior to hybridization with the complementary sequence associated to a supported lipid bilayer.[1,4–6] Such a system has been demonstrated to be highly specific since the self-assembled structures do not associate with the supported lipid bilayer unless the complementary sequence is present.[1] Besides, the composition and length of the nucleotide sequence can further be optimized to finely tune the hybridization driven surface immobilization of the liposomes,[4] which is thus more versatile than recognition between avidin and biotin.[7] We recently synthesized an amphiphilic DNA-*block*-copolymer, which self-assembles in vesicular structures in dilute aqueous solution. Given their larger molecular weight and therefore slower dynamics than their lipid analogues,[8] the structures resulting from the self-assembly of these oligonucleotide-polymer hybrids exhibit higher mechanical stability.[9] The surface-immobilization of the vesicular structures self-assembled from amphiphilic DNA-*block* copolymers thus appears as an elegant strategy to prepare soft, mechanically stable coatings. We demonstrate herein that the fine balance between non-covalent yet highly specific and directional hydrogen bonding between complementary nucleotide sequences enables the conformal immobilization of vesicular structures self-assembled from oligonucleotide-polymer hybrids. Poly(isobutylene)-*block*-oligonucleotide amphiphilic block copolymers self-assemble into vesicular structures in dilute aqueous solution.[10] The nucleotide sequence remains functional subsequent to polymer modification and self-assembly. Combining measurements with the quartz crystal microbalance (with dissipation monitoring, QCM-D), confocal laser scanning fluorescence microscopy (CLSM), and atomic force microscopy (AFM) we demonstrate that the nucleotide sequence involved in the self-assembly efficiently undergoes hybridization leading to full coverage of the surface with vesicular structures that retain their morphology upon surface immobilization through hybridization. The bacterial response appears to be sensitive to the softness of the coating since colonization is prevented on the surface-tethered vesicle layer resulting from the immobilization of self-assembled polymer-oligonucleotide hybrids.

Dr. N. Cottenye, Dr. K. Anselme, Dr. L. Ploux
Institut de Science des Matériaux de Mulhouse
(IS2M, CNRS LRC 7228)
15 rue Jean Starcky, BP 2488,
68057 Mulhouse Cedex, France
Dr. N. Cottenye, Prof. C. Vebert-Nardin[+]
Department of Chemistry, University of Basel
Klingelbergstrasse 80, CH-4056 Basel, Switzerland
E-mail: Corinne.vebert@unige.ch
[+] Present address: Department of Inorganic and Analytical Chemistry University of Geneva
Quai Ernest-Ansermet, 30 CH-1211 Geneva 4, Switzerland

**DOI: 10.1002/adfm.201200988**

## 2. Results and Discussion

Since it self-assembles in vesicular structures, the oligonucleotide-polymer hybrid used for these investigations is poly(isobutylene)-*block*-5′-GGGAGAGAGAGA-3′ ($PIB_{31}$-$G_7A_5$). Comprehensive synthesis and characterizations have been reported in a previous publication.[11] Briefly, this oligonucleotide-based amphiphilic diblock copolymer self-assembles in dilute aqueous solution into sub-micrometer size vesicular

structures (ca., 160 nm). We demonstrated as well, through circular dichroism, that the nucleotide sequences involved in the self-assembly retain their functional B-form chain configuration subsequent to polymer modification and self-assembly. In order to immobilize the vesicles self-assembled from the polymer modified nucleotide sequences, we used a surface-tethering of nucleotide sequences, which enables the achievement of optimal hybridization efficiency.[12–14] In brief, real time monitoring of the sequential steps of the grafting process was carried out combining the QCM-D with a wavelength interrogated optical sensor (BrightReader, Dynetix AG, Landquart, Switzerland)[13] to assess efficient grafting of the nucleotide sequences on the quartz crystals. Owing to the optimal grafting density of the surface-tethered nucleotide sequences, almost 100% hybridization efficiency with the complementary nucleotide strand could be demonstrated. The surface tethering displays stability against liquid flow due to the covalent attachment. In the present investigations, the surface was therefore modified accordingly with the strand complementary to the one involved in the self-assembly of the vesicles resulting from the structure formation of polymer-modified nucleotide sequences, i.e., 5′-TCTCTCTCTCCC-3′ ($C_7T_5$) tethered at the 5′ end.

Subsequent vesicle immobilization through hybridization with the surface-tethered nucleotide sequences was followed with the QCM-D. Upon injection of a solution of self-assembled $PIB_{31}$-$G_7A_5$, frequency and dissipation shifts due to the vesicle immobilization could be monitored (**Figure 1**).

A significant and rapid mass deposition as assessed by a decrease of the frequency shift down to -35 Hz associated with an increase of the monitored dissipation shift up to $2 \times 10^{-6}$ a.u. could be observed upon the deposition of the vesicles on the surface, which is characteristic of conformal immobilization. A decrease of the dissipation shift correlated to an increase of the frequency shift would be observed if disruption of the vesicular morphology were to occur in the time course of the quartz crystal oscillation.[15] Those would indeed correspond to mass and dissipation losses upon rupture and water release from the inner aqueous pool of the vesicles and formation of a layer of polymer-modified nucleotide sequences.[9,15] As can be observed in Figure 1, the deposited layer of vesicles is stable to rinsing with water, which assesses the strength of the immobilization of the amphiphilic DNA *block*-copolymer vesicles through hybridization. At full coverage, a dense layer of low average roughness of 3 ± 2 nm is achieved as assessed by AFM (**Figure 2**).

In order to quantify the deposited mass, we used the Voigt-based model, since significant dissipative properties of the layer prevents the use of the Sauerbrey equation.[15] An average mass of 630 ± 40 ng cm$^{-2}$ is deposited, which is somewhat lower than the theoretical value (812 ng cm$^{-2}$) at full coverage. However, slight heterogeneity in the size of the vesicles (ca., 20%, i.e., ± 163 ng cm$^{-2}$) might affect the theoretical mass determination.

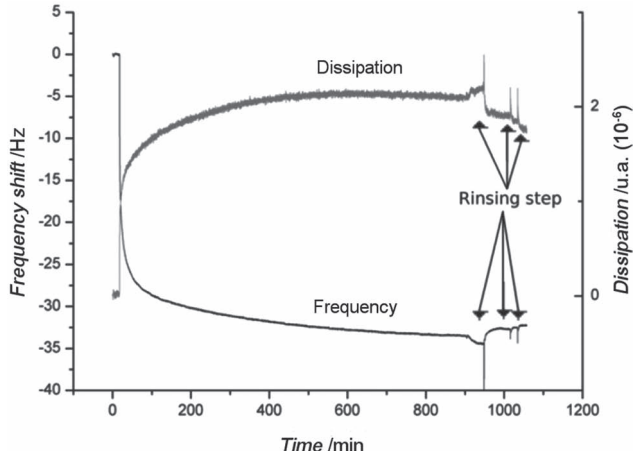



**Figure 1.** QCM-D monitoring of the immobilization of vesicles resulting from the self-assembly of oligonucleotide-polymer hybrids on a surface modified with the complementary sequence of the one involved in the self-assembly. The black curve corresponds to the frequency shift monitoring, whereas the gray curve displays the time course of the dissipation shift induced by the immobilization of vesicles onto the surface.

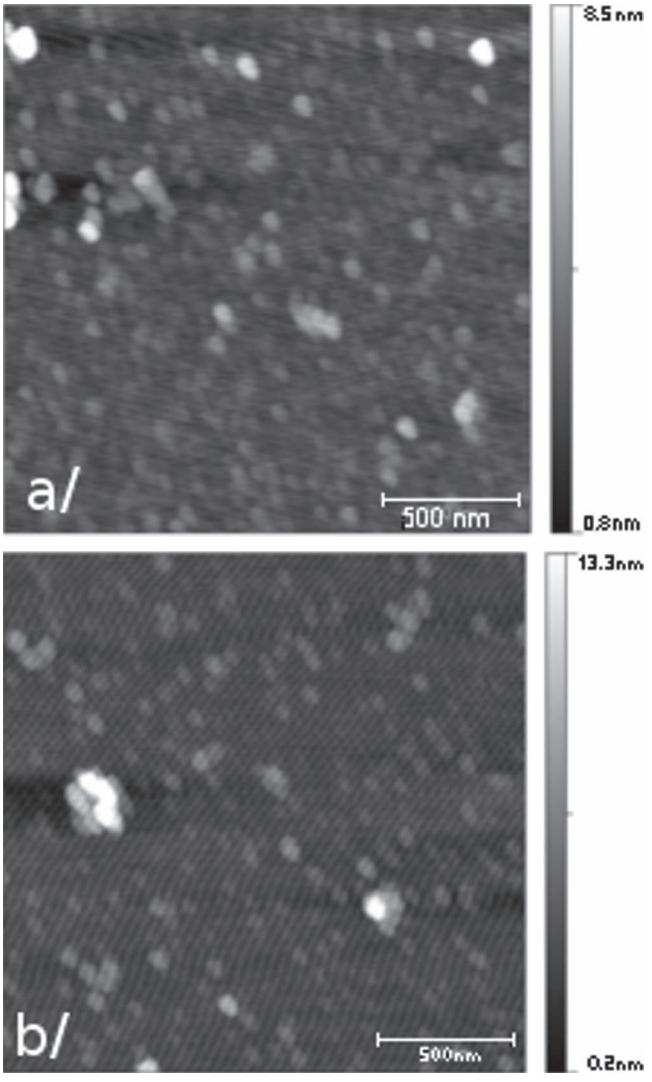



**Figure 2.** AFM of $PIB_{31}$-$G_7A_5$ vesicles hybridized on surfaces modified with the complementary strands. Full coverage of the surface by vesicles (a) and presence of aggregates of vesicles dispersed onto the surface (b) are observed.

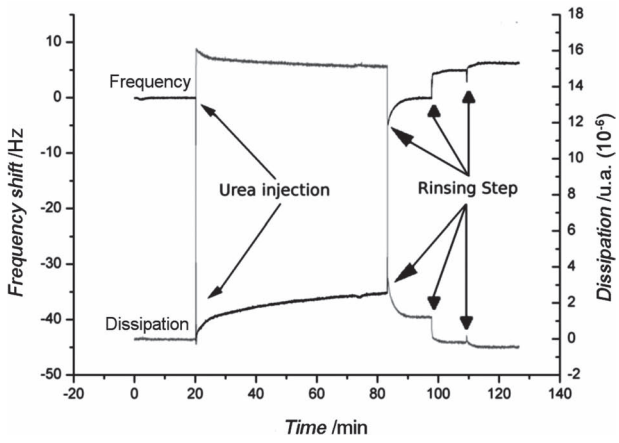



**Figure 3.** QCM-D monitoring of the effect of incubation with a 4 M urea aqueous solution of vesicles resulting from the self-assembly of polymer-modified nucleotide sequences immobilized through hybridization with the surface-tethered complementary strands. The black curve corresponds to the frequency shift monitoring, whereas the gray curve displays the dissipation decrease upon vesicle removal from the surface due to de-hybridization

The Voigt-based model further allows quantifying the average dissipative properties of the deposited layer. The layer of vesicles is characterized by a viscosity of 3.8 mPa s and a shear modulus of 0.6 MPa, being these values representative of a soft visco-elastic layer as compared to that of a control silicon wafer of about 51 GPa.[16]

In order to assess the strength of vesicles tethering to the surface through hybridization, we subsequently compared the stability of immobilization through the monitoring of the frequency and dissipation shifts after rinsing with either water or an aqueous solution of urea (4 M), which is known to de-hybridize double stranded nucleotide sequences (**Figure 3**).

In contrary to water rinsing, an increasing frequency shift was observed after injection of the urea aqueous solution, demonstrating that a part of the total mass was removed from the surface, which did not occur upon rinsing with water. The desorbed mass, calculated with the Voigt model, is of about 130 ± 10 ng cm$^{-2}$, which evidences partial de-hybridization of the vesicles (i.e., 20% of the initial mass of tethered vesicles). This low efficiency of de-hybridization is probably due to the diffusion limited access of urea to the surface tethered nucleotide sequences involved in the immobilization of the vesicles through hybridization, which results in the preparation of a rather stable coating.

To further assess the stability of the vesicle layer tethered on the surface by hybridization we followed this process in real-time with fluorescently labeled self-assemblies through the immobilization of a hydrophobic fluorescent dye within the vesicles membrane (Nile Red $\lambda_{ex}/\lambda_{em}$: 561/600–650 nm). To enable observation through CLSM (Zeiss CLSM LSM700), the solution of vesicular structures was not filtered prior to surface immobilization to not remove larger self-assemblies visible by light microscopy (**Figure 4**), leading to the deposition of large aggregates of vesicles.

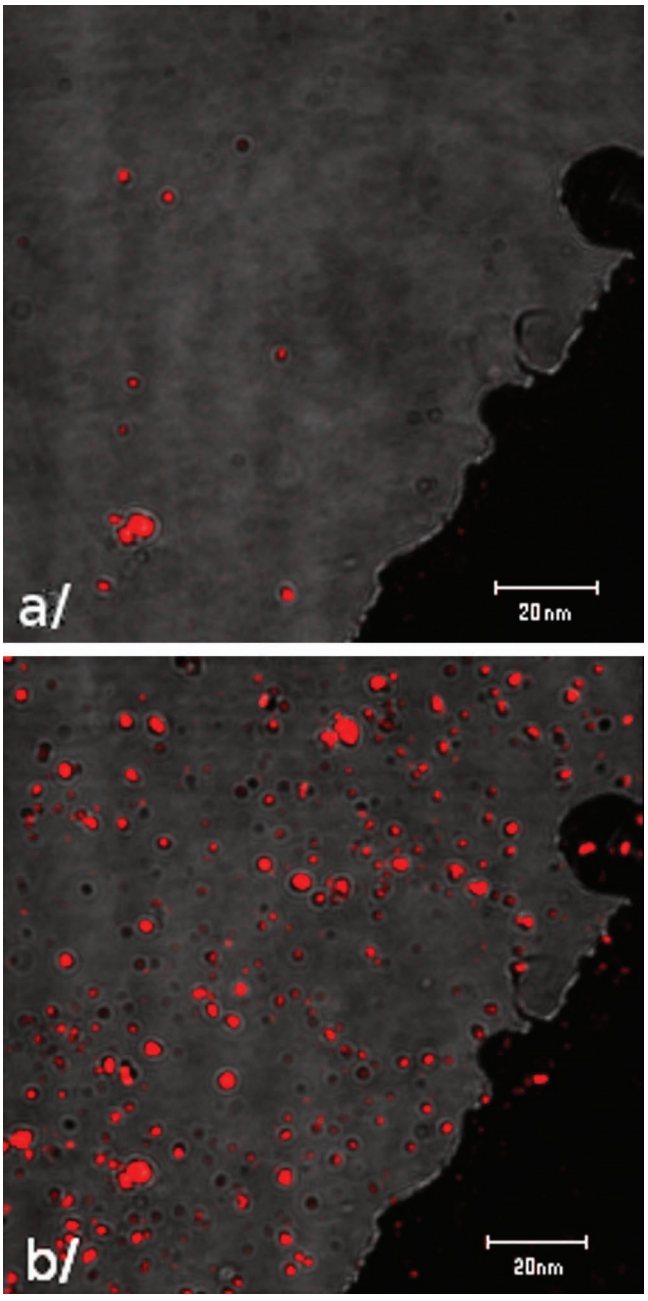



**Figure 4.** Fluorescence microscopy images obtained by CLSM of surface tethered $PIB_{31}$-$G_7A_5$ vesicles hybridized with their surface tethered complementary strands a) after 5 min of incubation and b) after 120 min.

Through scanning every 2 min during 2 h in the liquid static mode (**Figure 5**), it has been possible to follow in real time the hybridization of the larger structures self-assembled by the DNA block-copolymers, which was over after about 2 h, as already revealed by QCM-D monitoring (see Figure 1). Under flow (peristaltic pump flow of 18 mL h$^{-1}$), the larger self-assembled structures remained anchored at the surface and retained their morphology (Supporting Information: Movie 1). Few of the larger structures were eliminated by the flux, which further

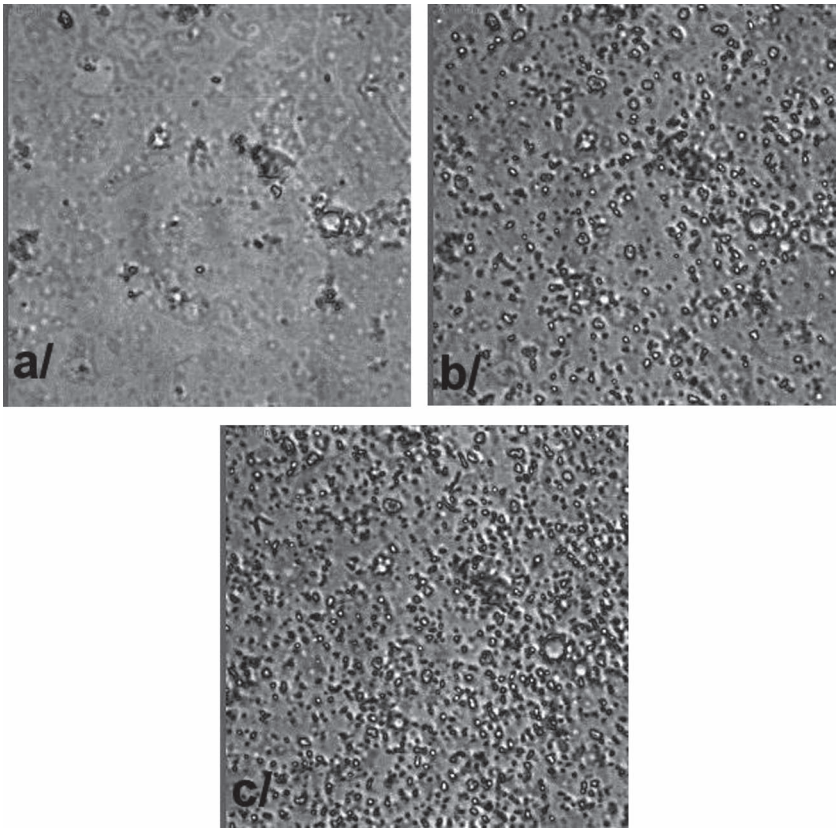



**Figure 5.** Immobilization on surfaces of vesicles self-assembled from polymer-modified nucleotide sequences through hybridization with the surface tethered complementary sequence with the one involved in the self-assembly in the flow cell under static condition at a) 0 min, b) 120 min, and c) 200 min.

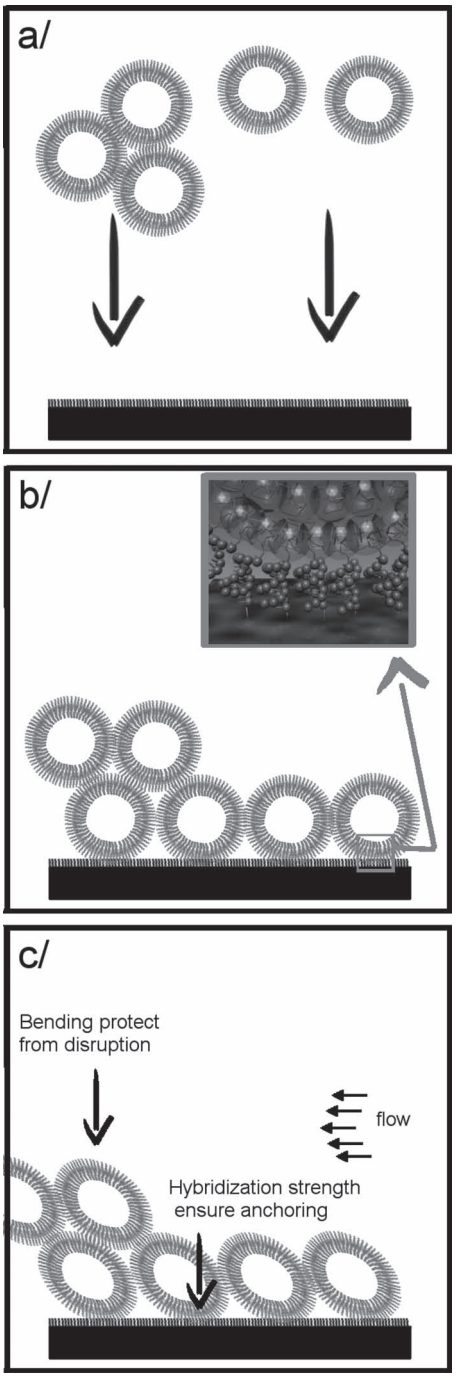
assesses the strength of immobilization through hybridization. More interesting is the dissipative property of the vesicular layer, which waves along with the flow induced by the peristaltic pump. The larger structures bend in the direction of the flow, without leaving their original position, being their shape restored when the flow stress was interrupted. The entire process of immobilization through hybridization can thus be described as depicted in **Scheme 1**.

Although some publications, yet few, evidence the possibility to control bacterial adhesion by tailoring the material softness,[17–20] the potential impact of surface mechanical properties on bacterial behavior and colonization on material is usually neglected and almost completely unknown. We report in here the effect of a dissipative surface on bacterial colonization, the soft coatings resulting from the surface tethering through hybridization of the DNA block-copolymer vesicular structures.

The soft surfaces were very efficient to avoid bacterial colonization in comparison to analogous surfaces of comparable chemistry and topography: i) The number of adherent bacteria adhered on soft coatings was low in comparison to that on control surfaces (silicon wafer modified with surface-tethered nucleotide sequences of similar composition than the one involved in the self-assembled DNA block-copolymer) (**Figure 6**). ii) As shown in Figure 6b, the few bacteria that adhered on vesicle-based coatings failed to proliferate on the surface when compared to what happened on the stiff control surface. Effects of surface chemistry and topography on these bacteria behaviors can be ruled out since these properties are comparable. The two types of surface reveal nucleotide sequences that are of the same composition, and vesicles-based surfaces slightly differ in their roughness ($3 \pm 2$ nm compared to $0.3 \pm 0.2$ nm), which was shown to not induce any significant difference in bacterial responses (**Figure 7**). This bacterial response should thus be attributed to the surface mechanical properties solely. Reduction of both adhesion and proliferation of bacteria therefore allows considering that tailoring the surface mechanical properties is an approach to control not only bacterial adhesion but even proliferation of already adhered bacteria.

Further investigations of the bacterial behavior on soft vesicle-based coatings suggested that proliferation of the few number of adherent bacteria occurred, however without allowing the retention of daughter bacteria on the surface (real time observation, Supporting Information: Movie 2). Bacterial displacement length and speed were therefore estimated, and compared to displacement length and speed observed on the control surface (silicon wafer). Although speeds were similar on vesicle-based coatings as well as on control surfaces ($0.1 \pm 0.5$ and $0.1 \pm 0.1$ $\mu m\ s^{-1}$ respectively), the high standard deviation of the speed value quantified on vesicle-based coatings highlighted the high variability of the bacterial response. This behavior probably results from the presence on the surface of two bacteria populations: as shown in **Figure 8**, a first part of adherent bacteria revealed displacement lengths similar to those observed for bacteria adherent to control surfaces, while a second part was moving on the surface, sometimes reaching displacements of almost 10 μm and even detachment from the surface. This may finally explain why adherent bacteria failed to colonize the vesicle-based surface, as already evoked and shown in Figure 6.

Even if surface mechanical properties are considered as a critical factor in modulating eukaryotic cell adhesion, or the function and differentiation of stem cells,[21–23] only few publications have reported the influence of surface mechanical properties on bacterial adhesion. Van der Mei and co-workers[19] as well as Lichter et al.[17,18] have demonstrated positive correlations between the increase of both the surface elastic modulus and rate of bacterial adhesion. However, van der Mei and co-workers highlighted the impossibility to get the correlation in specific flow conditions (parallel plate flow chamber versus stagnation point flow chamber), suggesting the complex influence of the coupling of the surface mechanical properties with the surrounding conditions on bacterial behavior. Our results confirm the effect of surface mechanical properties on bac-




**Scheme 1.** A surface modified with the complementary nucleotide sequence to the one involved in the self-assembly is immersed in a solution of DNA *block*-copolymer vesicles (a), hybridization rapidly occurs (b), leading to surface full coverage. Vesicle bending due to peristaltic flow occurs. Immobilization through hybridization ensures anchorage (c). The mechanical stability and fluidity of the oligonucleotide-based *block* copolymer membrane allow vesicles bending without disruption.

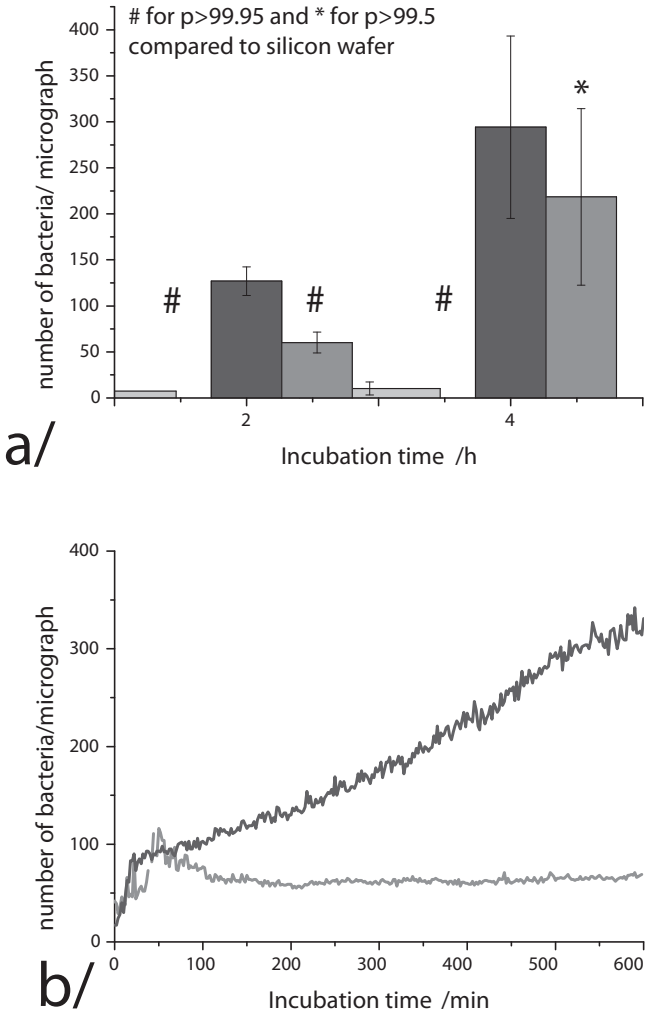



**Figure 6.** a) Quantity of adherent bacteria on soft vesicle-based coatings (light gray bars) compared to adhesion on naked silicon wafer (black bars) and silicon wafer grafted with a nucleotide sequence of the same composition than the one involved in the self-assembly of DNA *block*-copolymer vesicles ($A_5G_7$, gray bars). b) Evolution with time of the number of adhering bacteria on soft vesicle-based coatings (gray line) and naked silicon wafer (black line).

terial colonization. They also allow specifying that not only bacterial adhesion but also bacteria proliferation are affected, through a probable correlation between surface mechanical properties and bacterial motility (i.e., bacteria displacement on surfaces). Moreover, the results of the studies reported in here suggest that both the compressive contribution and shear or/ and viscosity contributions of the surface mechanical properties may play a role in the behavior of bacteria. This hypothesis is further supported by the results of preliminary studies performed in static culture mode (Supporting Information Figure 2), which evidence that the number of adherent bacteria increases with the concentration of an agar substratum. On surfaces prepared at low agar concentration, i.e., low Young modulus, colonization is prevented. These results are in agreement with the low colonization observed on the soft surfaces based on vesicles of low elasticity, assessing a noticeable link between the cell response and the elasticity of the surface. We therefore hypothesize that i) bacterial behavior in response

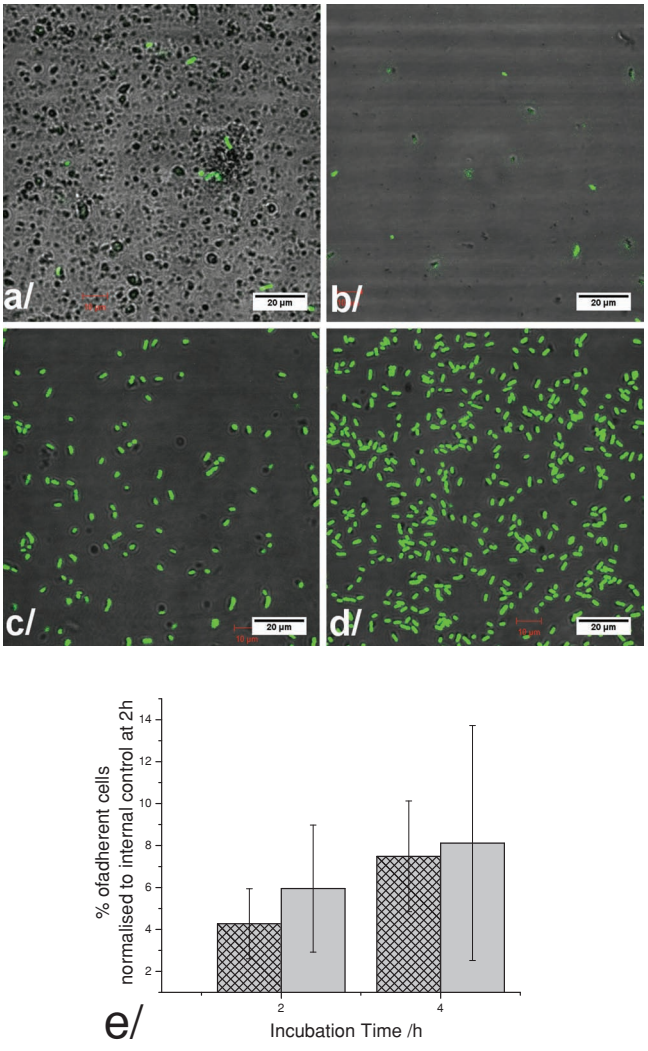



**Figure 7.** Confocal fluorescence imaging, combining reflection and fluorescence, of bacteria adhered onto the different surfaces: a) soft vesicle-based coatings with rough topography (Ra = 20 ± 2 nm), b) soft vesicle-based coatings with smooth topography (Ra = 3 ± 2 nm), c) silicon wafer grafted with $A_5G_7$ nucleotide sequences, and d) reference naked silicon wafer. e) Comparison between the number of adhered bacteria on soft vesicle-based coatings with rough topography (black sparse grey bars) and soft vesicle-based coatings with smooth topography (grey bars) at 2 and 4 h of incubation, normalized to the number of adherent bacteria on naked silicon wafer at 2 h.

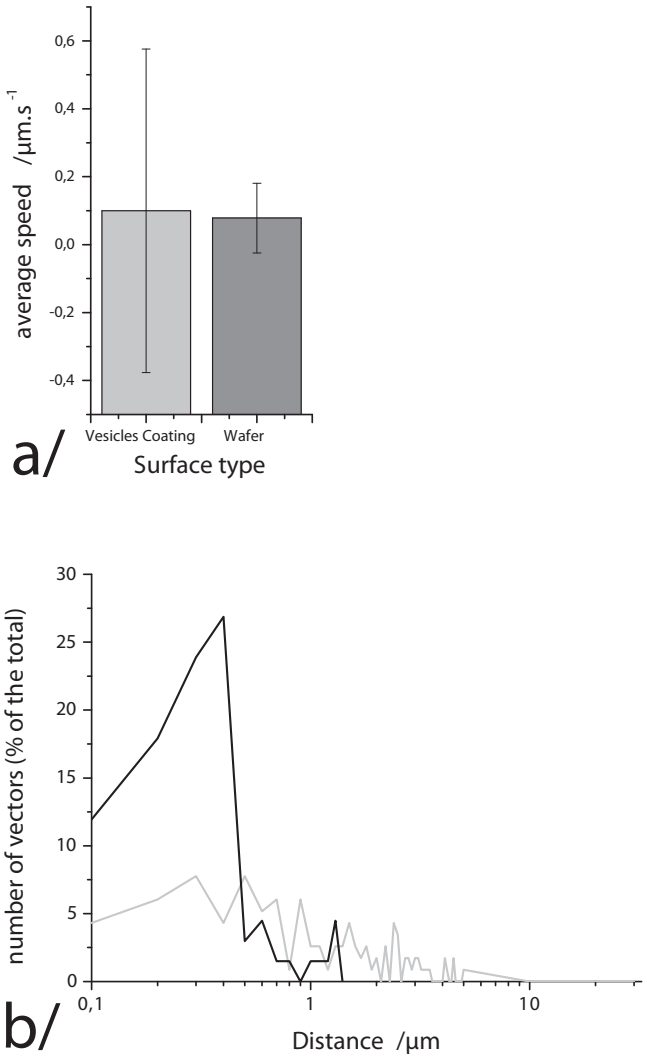



**Figure 8.** a,b) Comparison of displacement of bacteria on vesicle-based surfaces (light gray line) and stiff silicon wafer surfaces (black line). The displacement of each bacterium was tracked over the incubation time, being each of these displacements translated in a displacement vector. A displacement smaller than 1 µm (smaller than the bacterial size) indicates a simple reorientation of the bacteria, while a displacement greater than 1 µm highlights a change of bacterial location on the surface (re-localization).

to surface mechanical properties is complex and probably depends on various mechanical parameters corresponding to several components of the mechanical properties (compressive, tensile, and shear moduli, viscosity, elasticity and anisotropic properties arising from the impenetrable wall effect due to the presence of an interface); ii) coating–related parameters like coating hydration, coating thickness and structure, and polymer chain confinement due to the elaboration process, as well as surrounding properties such as hydrodynamic conditions may influence the bacterial response to the above mechanical parameters.

## 3. Conclusions

In the present investigations we demonstrate for the first time the possibility to immobilize DNA-block-polymer vesicles through hybridization, being the constitutive hydrophilic block of the self-assembling amphiphile a nucleotide sequence complementary to the surface-tethered strand. This fast and spontaneous process leads to conformal immobilization of the self-assembled structures. Owing to immobilization through hybridization, mechanical stability as well as fluidity of the DNA block-copolymer membrane, the resulting coating is stable to hydrodynamic stress, which enabled conducting investigations

of the cellular response to this substrate under static and flow modes. The obtained dissipative layer prevents bacterial colonization, being the origin of the prevention of this bacterial adhesion mechanical rather than chemical or topographical. Exploiting surface mechanical properties for preventing surface colonization by bacteria thus appears as a new promising future strategy for the prevention of biofilm formation and biofilm-related infections.

## 4. Experimental Section

*Material*: 3-aminopropyltriethoxysilane 99% (APTES), anhydrous toluene 99%, glutaraldehyde 25% solution grade II, propylamine 98%, formamide were all purchased from Sigma-Aldrich, Switzerland. Chloroform 99% was purchased from J. T. Baker, Switzerland. Sodium cyanoborohydrate was purchased from Fluka, Switzerland. Amino-modified oligonucleotides: $(C_7T_5)$-$NH_2$ and $(A_5G_7)$-$NH_2$ were purchased from Mycrosynth (Balgach, Switzerland). Silicon wafers single side polished, orientation N/Phos<100>, 525 ± 25 µm thickness, 1-30 Ohm $cm^{-1}$ resistivity were purchased from Si-Mat Silicon Materials, Landsberg am Lech, Germany. Doubly distilled water was used for the preparation of all samples.

*Methods- Surface Tethering of Nucleotide Sequences*: Surfaces of high grafting density were prepared with the protocol published in a previous work,[12] but differed at the quenching step. Sodium cyanoborohydrate was used to reduce the Shiff-base formed between the amino-termination of the oligonucleotide and the aldehyde moiety revealed at the surface.[24] With this reduction, the linkage becomes irreversible and the grafted oligonucleotides are protected from detachment during de-hybridization.

*Vesicles Labeling*: Labeled vesicles were prepared by mixing 0.3 mg of $PIB_{31}$-$G_7A_5$ bulk copolymer with 1 µL of a 50 µg $mL^{-1}$ Nile Red solution (Sigma-Aldrich) in DMSO into 1 mL of NaCl buffer (150 mM, pH 6.5). The solution was mixed in a dark flask during 4 h at room temperature and stored overnight at 4 °C. Vesicles purification was performed through size exclusion chromatography on a Sephadex G100 (GE-Healthcare) size exclusion chromatography gel. Since vesicle size ca. 160 nm does not allow resolution through confocal microscopy, the obtained solution was only filtered through 0.45 µm membrane filters, allowing the observation of large structures of self-assembled vesicles.

*Vesicles Immobilization*: Vesicle-coated surfaces were prepared by the incubation of a solution containing vesicular structures self-assembled from poly(isobutylene)-*block*-5′-GGGAGAGAGAGA-3′ ($PIB_{31}$-$G_7A_5$) in a well containing a surface coated with the complementary single stranded nucleotide sequence $C_7T_5$ (5′-TCTCTCTCTCCC-3′). After 12h of incubation at room temperature, the vesicle-coated surface, obtained by spontaneous hybridization, was rinsed three times with NaCl buffer (NaCl 150 mM) and sterilized 30 min under UV (180 nm) in NaCl buffer.

To obtain "smooth" coatings, the solution of vesicles obtained by bulk swelling was sequentially filtered 3 times through 0.45 µm and 3 times through 0.2 µm pore size filters to break the larger structures. This preparation allows obtaining surfaces of long term stability, with a smooth topography, and mechanical properties induced by the vesicular structures properties.

*Control Surfaces*: Control surfaces consisted of silicon wafers prepared as follow: they were cleaned 3 times in chloroform under sonication for 15 min. Then wafers were activated by 15 min UV treatment (UV/ozone cleaner, $\lambda = 180$ nm). Finally wafers were sterilized by a dried heat procedure (1 h, 180 °C).

*Quartz Crystal Microbalance with Dissipation QCM-D*: QCM-D investigations were performed with the Qsense Quartz Cristal Microbalance (QCM-D, Goteborg, Sweden) with QSX 303 silicon dioxide sensors. All data were extracted with the QTools software.[25]

*Atomic Force Microscopy (AFM)*: Tapping mode AFM analyses of the samples were performed using a PycoLE system, Molecular Imaging, with silicon nitride cantilevers, k = 42 N $m^{-1}$, scan rate 0.5 lines $s^{-1}$. Different locations of the samples were monitored. All experiments were done in air at room temperature.

*Fluorescence Microscopy*: An upright CLSM (LSM700–Zeiss, Germany) was used to follow time resolved vesicle deposition. Excitation at 405 nm was used in the reflection imaging mode. To observe Nile Red fluorescence,[26] excitation at 532 nm was used and emission was recorded at 550–630 nm, and images were taken every 2 min.

Fluorescence microscopy under flow was performed with the Upright CLSM (LSM700–Zeiss, Germany) in a home-designed flow cell. Subsequent to the flow cell mounting, as described below, the flow of NaCl buffer (150 mM, pH 6.8) was started, left during two hours and the surface imaged overtime (every 2 minutes). Flow was then stopped and 5 mL of a solution of fluorescently labeled vesicles (Nile Red) was injected in the flow cell without flow. Labeled vesicles and surfaces were incubated without flow for 12 h. Images were still taken every 2 min in reflection and fluorescence modes. Then medium flow was turned on for 2 h under imaging.

*Bacterial Strains*: Experiments were conducted with the green fluorescent *Escherichia coli* strain SCC1 modified with a green fluorescent protein (GFP) genetic insert.[27]

*Culture Condition*: Bacteria were cultivated in a selective M63 medium supplemented with glucose (0.2%) (pH 6.8) at 30 °C.[28] Prior to each experiment, bacteria were grown for 14 h in fresh medium. This culture was used to inoculate a second pre-culture (10 vol.-% of the first pre-culture) which was grown for 4 h before inoculating the final culture (10 vol.-% of second pre-culture) for an $OD_{630} = 0.01$.

*Investigation of the Bacterial Response in Static Conditions*: The substrates were placed in 6-well plates and 4 mL of fresh bacterial culture was added to each well. Bacteria were grown on the substrates at 30 °C. Two incubation times were explored: 2 h, 4 h. One series consists of two substrates of each type (i.e. surface tethered nucleotide sequences, soft vesicle-based coatings) for each incubation time, another of control substrates (silicon wafer).

After incubation, the samples were rinsed three times to eliminate non-adherent bacteria. For this purpose, 3 mL of supernatant, i.e. bacterial growth medium for the first rinsing and NaCl solution for the subsequent washings, were removed and replaced with a fresh NaCl solution (9 g $L^{-1}$ in water).

*Cytotoxicity of the Surface*: In order to confirm the non-toxicity of the surface on planktonic bacteria, the absorbance at 630 nm ($A_{630nm}$) of each culture (supernatant from each well) was measured and compared to growth on the control surface.

*Biofilm Observation and Fluorescence Staining*: Confocal microscopy was used for observation (Zeiss, LSM700). Biofilms were observed directly after the last rinsing step using a 9.1 mm working distance focal objective (Zeiss LD EC "epiplanneofluar" 50X/055 DIC M27). The laser wavelength used for excitation was 405 nm for imaging in the reflection mode. The fluorescence of the GFP expressed by SCC1 was exploited for imaging in the fluorescence mode. A laser wavelength of 488 nm was used for excitation, while collection of the emission fluorescence was done in the 420–550 nm range. Two surfaces of each type were used for each experiment and each surface was imaged at 10 different locations randomly chosen.

*Analysis of Biofilm Confocal Microscopy Images*: Image processing was performed using the Image J V.1.44a software with the LSM toolbox V4.0g plugging,[29] allowing separation of fluorescence and reflection images from Zeiss LSM raw data. Then, extracted images were processed with the CellC software[30] for counting the number of adherent bacteria.

*Quality Control*: The results are expressed in terms of mean values and standard deviations for two samples of each substrate type, including control. Significant differences between sample sets were determined using the classical parametric test for mean comparison, the so-called Student test.

*Investigation of the Bacterial Response in Dynamic Conditions Flow Cell Design*: A specific flow cell was designed for experiments conducted under a Zeiss LSM700 confocal microscope (Supporting information Figure 1). Flow cell was made with the support of the Technical University of Denmark, Lyngby, Denmark.[31]

The flow cell design allows having a peristaltic laminar flow at the surface of the sample (tested on 1 mm thick silicon wafer). The chamber has a volume large enough to avoid any border effect (reduced flow speed or non-laminar flow) of the flow at the surface. The entire setup consists of a flow cell connected with Tygon tubings (ø 1.52 mm) to a peristaltic pump (Ismatech IP multichannel peristaltic pump) with an intermediate bubble trap (designed by the Technical University of Denmark, Lyngby, Denmark).[31] Medium and trash bottles are closed with auto-cleavable caps equipped with filters (0.2 µm, Millipore). A three ways connector was used to allow injection.

*Bacterial Experiment Under Flow*: The two parts of the flow cell are connected together with silicone rubber (Soudalaquarium) prior to sterilization of all parts of the setup (flow-cell, tubing, connectors and bottles). The complete setup was mounted under sterile conditions in a laminar flow hood. Subsequently, the sterile surface is placed in the center of the flow cell, which is then closed by sealing the glass cover slip with an external joint of silicon rubber (Soudalaquarium).

At first, the whole setup is installed under the LSM 700 confocal microscope and the culture medium (M63G) is flowed for 2 h with a flow rate of 18 mL $h^{-1}$. Then, the pump is turned off. 5mL of 0.1 $OD_{630}$ bacterial cultures (obtained by a dilution of overnight culture) is injected into the cell and incubated 20 min without flow at room temperature. During that time, the surface focal plane is imaged with the confocal microscope. The medium is then flowed at 18 mL $h^{-1}$ and pictures are taken every 2 min during 10 h. Both fluorescence and reflection modes are used as described above for fluorescence microscopy.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Acknowledgements

The SNSF (200020-121822 and PPOOP2-128380) and NCCR-NANO are greatly acknowledged for financial support. Christian Gauthier and Damien Favier are acknowledged for the measurements of agar coating mechanical properties.

Received: April 6, 2012
Revised: June 2, 2012
Published online: July 20, 2012

[1] C. Yoshina-Ishii, S. G. Boxer, *J. Am. Chem. Soc.* **2003**, *125*, 3696.
[2] A. Gunnarsson, P. Jonsson, R. Marie, J. O. Tegenfeldt, F. Hook, *Nano Lett.* **2008**, *8*, 183.
[3] G. Stengel, R. Zahn, F. Höök, *J. Am. Chem. Soc.* **2007**, *129*, 9584.
[4] Y.-H. M. Chan, P. Lenz, S. G. Boxer, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 18913.
[5] M. Chung, R. D. Lowe, Y.-H. M. Chan, P. V. Ganesan, S. G. Boxer, *J. Struct. Biol.* **2009**, *168*, 190.
[6] M. M. Lozano, C. D. Starkel, M. L. Longo, *Langmuir* **2010**, *26*, 8517.
[7] B. Städler, D. Falconnet, I. Pfeiffer, F. Höök, J. Vörös, *Langmuir* **2004**, *20*, 11348.
[8] A. Jesorka, O. Orwar, *Annu. Rev. Anal. Chem.* **2008**, *1*, 801.
[9] R. P. Richter, R. Bérat, A. R. Brisson, *Langmuir* **2006**, *22*, 3497.
[10] F. J. Teixeira, P. Rigler, C. Vebert-Nardin, *Chem. Commun.* **2007**, 1130.
[11] N. Cottenye, M.-I. Syga, S. Nosov, A. H. E. Müller, L. Ploux, C. Vebert-Nardin, *Chem. Commun.* **2012**, *48*, 2615.
[12] N. Cottenye, F. T. Jr, A. Ponche, G. Reiter, K. Anselme, W. Meier, L. Ploux, C. Vebert-Nardin, *Macromol. Biosci.* **2008**, *8*, 1161.
[13] J. Razumovitch, K. de França, F. Kehl, M. Wiki, W. Meier, C. Vebert, *J. Phys. Chem. B* **2009**, *113*, 8383.
[14] J. Razumovitch, W. Meier, C. Vebert, *Biophys. Chem.* **2009**, *139*, 70.
[15] R. Richter, A. Mukhopadhyay, A. Brisson, *Biophys. J.* **2003**, *85*, 3035.
[16] M. A. Hopcroft, W. D. Nix, T. W. Kenny, *J. Microelectromech. Sys.* **2010**, *19*, 229.
[17] J. A. Lichter, M. F. Rubner, *Langmuir* **2009**, *25*, 7686.
[18] J. A. Lichter, M. T. Thompson, M. Delgadillo, T. Nishikawa, M. F. Rubner, K. J. Van Vliet, *Biomacromolecules* **2008**, *9*, 1571.
[19] D. P. Bakker, F. M. Huijs, J. de Vries, J. W. Klijnstra, H. J. Busscher, H. C. van der Mei, *Colloids Surf. B* **2003**, *32*, 179.
[20] F. Boulmedais, B. Frisch, O. Etienne, P. Lavalle, C. Picart, J. Ogier, J.-C. Voegel, P. Schaaf, C. Egles, *Biomaterials* **2004**, *25*, 2003.
[21] L. Richert, A. J. Engler, D. E. Discher, C. Picart, *Biomacromolecules* **2004**, *5*, 1908.
[22] M. T. Thompson, M. C. Berg, I. S. Tobias, M. F. Rubner, K. J. Van Vliet, *Biomaterials* **2005**, *26*, 6836.
[23] D. E. Discher, P. Janmey, Y. Wang, *Science* **2005**, *310*, 1139.
[24] S. Onclin, B. J. Ravoo, D. N. Reinhoudt, *Angew. Chem. Int. Ed.* **2005**, *44*, 6282.
[25] I. Reviakine, F. F. Rossetti, A. N. Morozov, M. Textor, *J. Chem. Phys.* **2005**, *122*, 204711.
[26] W. Mueller, K. Koynov, K. Fischer, S. Hartmann, S. Pierrat, T. Basché, M. Maskos, *Macromolecules* **2009**, *42*, 357.
[27] H. Miao, S. Ratnasingam, C. S. Pu, M. M. Desai, C. C. Sze, *J. Microbiol. Meth.* **2009**, *76*, 109.
[28] C. Prigent-Combaret, E. Brombacher, O. Vidal, A. Ambert, P. Lejeune, P. Landini, C. Dorel, *J. Bacteriol.* **2001**, *183*, 7213.
[29] W. S. Rasband, *ImageJ*, U. S. National Institutes of Health, Bethesda, MD **1997**.
[30] J. Selinummi, J. Seppälä, O. Yli-Harja, J. Puhakka, *Biotechnology* **2005**, *39*, 859.
[31] *Current Protocols in Microbiology*, (Eds: R. Coico, T. Kowalik, J. Quarles, B. Stevenson, R. Taylor), John Wiley & Sons, Inc., Hoboken, NJ **2005**.