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A General Approach to the Covalent Immobilization of Single Polymers**

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Nano- and molecular-scale materials and devices have demonstrated great potential for unique properties and functions that are unattainable at macroscopic scales.^[1] Studies of single molecules and their interactions and reactions at the single-molecule level provide new perspectives on important fundamental issues in physics, chemistry, and biology.^[2] Often it is desirable to anchor the molecules on suitable substrates to facilitate the study and application of single molecules under various conditions for physical measurement and chemical processing. Two common approaches to single-polymer immobilization are electrostatic adsorption and chemical grafting. The first method involves the deposition of a very dilute polymer solution on a substrate followed by evaporation of the solvent. A key requirement is that the polymer and the substrate possess opposite charges under the experimental conditions for the electrostatic interactions to occur.^[3–5] The second method involves chemically grafting the polymer to the substrate. In this case, the polymer is derivatized with a functional group that is subsequently conjugated to the substrates. Examples include Cl-terminated poly(dimethylsiloxane) on silicon,^[6] disulfide-modified polystyrene on gold,^[7] and polysilanyl lithium on a brominated quartz surface.^[8] Relative to noncovalent and electrostatic interactions, covalent immobilization offers more stable and robust attachments that can withstand harsh experimental conditions. Herein, we report a general approach to the covalent immobilization of single polymer molecules. This method does not require chemical derivatization of the polymer and is applicable to a variety of materials, including those that do not possess reactive functional groups.

The immobilization chemistry is based on the photochemically or thermally initiated C—H/N—H insertion reactions of perfluorophenylazides (PFPA)s.^[9–11] The presence of fluorine atoms markedly increases the insertion reaction yield relative to their non-fluorinated counterparts.^[12] In the example shown in Scheme 1, a glass or silicon wafer is first treated with PFPA–silane, thus attaching azido groups to the surface. The polymer is

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then coated and the azido groups activated to form covalent linkages to the polymer. We found that this immobilization method is highly reproducible and tolerant of defects. Uniform polymer films were obtained from wafers treated with PFPA–silane at concentrations of a few micromoles, or when more than 100 times of a non-photoactive silane was added,^[13] which is because in principle, only one attachment point is necessary to tether the entire polymer chain to the surface. We therefore hypothesize that as the density of the surface azido groups continues to decrease, the immobilized polymer will no longer be uniform. The thickness of the polymer film will decrease and eventually the surface will be populated by isolated single polymer molecules. The density of the surface azido groups can be controlled by the concentration of PFPA–silane or by treating the surface with a mixture of PFPA–silane and a non-photoactive silane.^[13]

Herein, we report the covalent immobilization of single polymer molecules using dilute PFPA–silane solutions. Comprehensive studies were conducted on polystyrene, a polymer that does not possess any functional groups. In the experiment, cleaned silicon wafers were treated with solutions of PFPA–silane in toluene, the concentrations of which ranged from 5×10^{-1} to 5×10^{-5} mg mL⁻¹. The wafers were then spin-coated with polystyrene. Irradiation followed by removal of the unbound polymer with toluene yielded covalently attached polymers. The thickness and water-contact angles of the resulting films are shown in Figure 1.

As the concentration of PFPA–silane decreased, the immobilized polymer became thinner (Figure 1 a). At the PFPA–silane concentration of 5×10^{-3} mg mL⁻¹, the contact angle decreased to approximately 57°, thus indicating the collapse of the film (Figure 1 b). Further dilution resulted in lower contact angles and no film was detected by ellipsometry. When the immobilization was carried out on wafers treated with PFPA–silane at 5×10^{-5} mg mL⁻¹, isolated single polystyrene molecules were observed (Figure 2 a).

To confirm that the particles were indeed polymer single molecules, polystyrene of various molecular weights was used. The size of a polymer is directly related to its molecular weight: the higher the molecular weight, the larger the radius of gyration (R_g) and thus the size of the molecule. Indeed, we observed that the size of the particles, that is, the immobilized single polymer molecules, increased with the molecular weight of the polymer (Figure 2).

We found that the higher the molecular weight, the lower the concentration of PFPA–silane was needed to obtain single-molecule immobilization (see Figure 2). This finding is consistent with the immobilization chemistry shown in Scheme 1. As the molecular weight and thus size of the polymer increases, less surface azido groups are needed to attach the polymer. The concentration of PFPA–silane was therefore lowered to observe isolated single molecules.

Atomic force microscopy (AFM) revealed that the immobilized polystyrene adopted a cone shape (Figure 3). The volume of a single polystyrene molecule V can thus be calculated according to Equation (1), where H is the height and D is the diameter of the molecule

measured by AFM. Results showed that the measured values (V) are in good agreement with those calculated from the molecular weights of the polymer (V_{calcd} ; Table 1).^[4]

$$V = 1/3 H \pi \left(\frac{D}{2} \right)^2 \quad (1)$$

The versatility of the method was tested with poly(2-ethyl-oxazoline) (PEOX), a hydrophilic polymer that also lacks reactive functional groups. Following a similar procedure, single molecules of PEOX were successfully immobilized (Figure 4). A much higher concentration of PFPA-silane was needed for PEOX ($5 \times 10^{-1} \text{ mg mL}^{-1}$) than for polystyrene ($10^{-5} \text{ mg mL}^{-1}$, $M_w = 570\,000$). This difference can be attributed to the difference in the immobilization efficiency of the two polymers, which is in part governed by the local concentration of the polymer in proximity to the azide-functionalized surface.^[14] Evaluation of 62 particles from the AFM image yielded the average particle volume of 1764 nm^3 ($D = (53 \pm 8) \text{ nm}$, $H = (2.4 \pm 1.1) \text{ nm}$), whereas the calculated molecular volume is 830 nm^3 based on the average molecular weight of the sample ($M_w = 500\,000$). Unlike polystyrene samples, which are monodisperse, the PEOX obtained from Aldrich is polydisperse (molecular weight distribution is unknown); a polydisperse polymer is more heterogeneous than its monodisperse counterpart with respect to molecular weight. When a polymer containing various sized molecules is coated on a surface with a limited amount of azido groups, larger molecules, that is, polymers of higher molecule weight, are more likely than smaller molecules to be attached. The molecular volume of the immobilized polymer is thus larger than the theoretical value that is calculated from the average molecular weight of the original sample. This preferential immobilization of larger sized polymers was also observed for polystyrene (Table 1), although the deviations were smaller than PEOX as a result of the monodisperse nature of the polystyrene samples.

Extended single polymer chains were occasionally observed as shown in Figure 5. The curvilinear length was measured to be 430 nm . This value falls in the range of $95\text{--}953 \text{ nm}$, as calculated from the molecular-weight distribution of the polystyrene sample.^[15–16]

In summary, a simple and general method has been developed for the covalent immobilization of single polymer molecules on silicon wafers. Because the immobilization is based on C—H/N—H insertion reactions, it is versatile and especially suited for molecules that do not possess functional groups and are difficult to be immobilized by other means. Single-polymer immobilization was also achieved by treating the wafer surface with a mixture of PFPA-silane and a non-photoactive silane. Studies are underway to investigate the nature of the attachment, including the polymer attachment point and orientation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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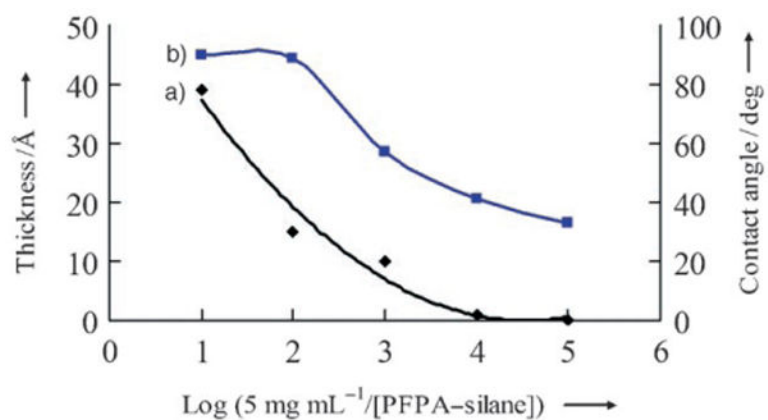


Figure 1. a) Thickness, measured by ellipsometry, and b) water contact angle of the immobilized polymer as a function of PFPA-silane concentration. The contact angle of the unfunctionalized silicon wafer was measured to be approximately 17°.

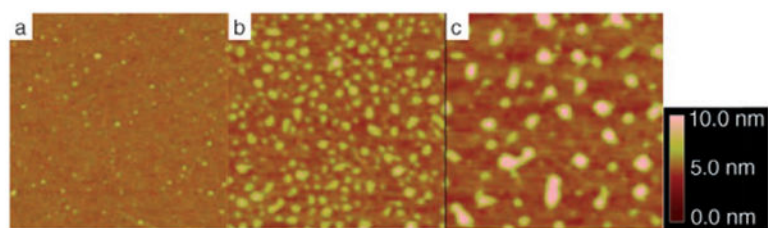


Figure 2. Single polystyrene molecules covalently immobilized on silicon wafers. Monodisperse polystyrene used: a) $M_w = 223\,200$, b) $570\,000$, and c) $1\,877\,000\text{ g mol}^{-1}$. The wafers were treated with PFPA–silane at concentrations of a) 5×10^{-5} , b) 1×10^{-5} , b) $5 \times 10^{-6}\text{ mg mL}^{-1}$, respectively. The scan area is $1 \times 1\ \mu\text{m}^2$ for all three images.

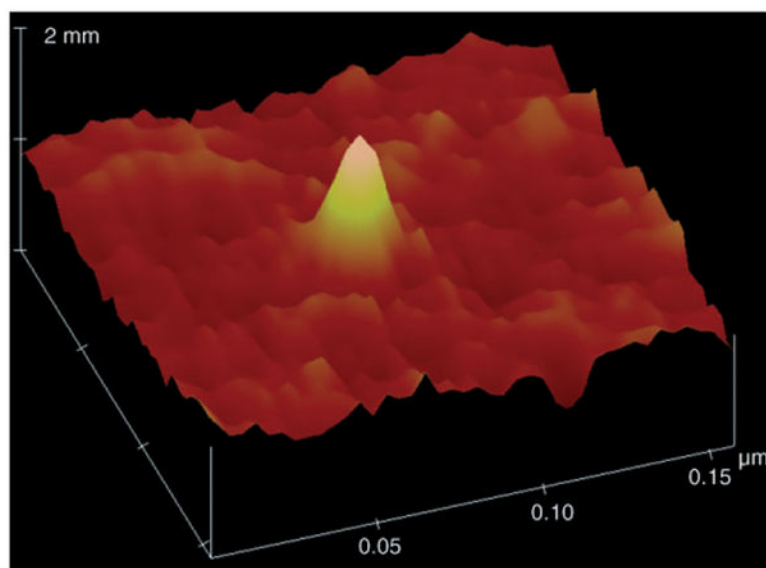


Figure 3.
AFM topographic image enlarged from Figure 2 a.

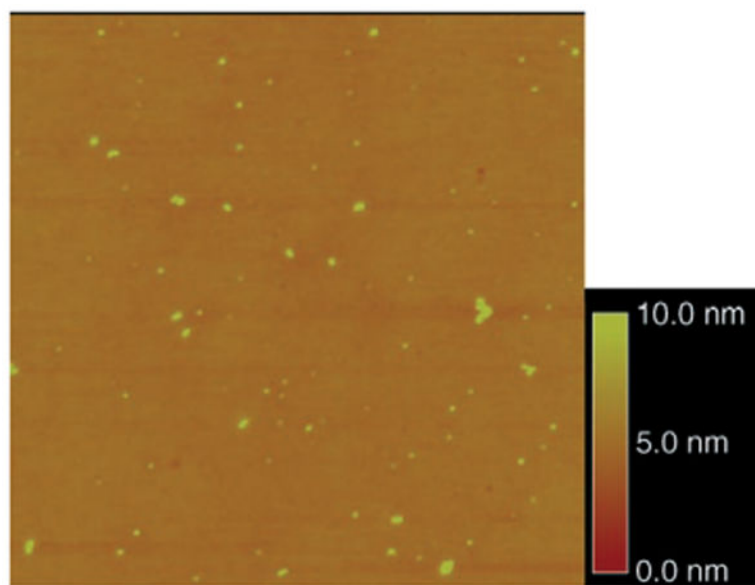


Figure 4. AFM topographic image of immobilized PEOX ($M_w = 500000 \text{ g mol}^{-1}$) on a silicon wafer treated with $5 \times 10^{-1} \text{ mg mL}^{-1}$ of PFPA-silane in toluene. The scan area is $3 \times 3 \mu\text{m}^2$.

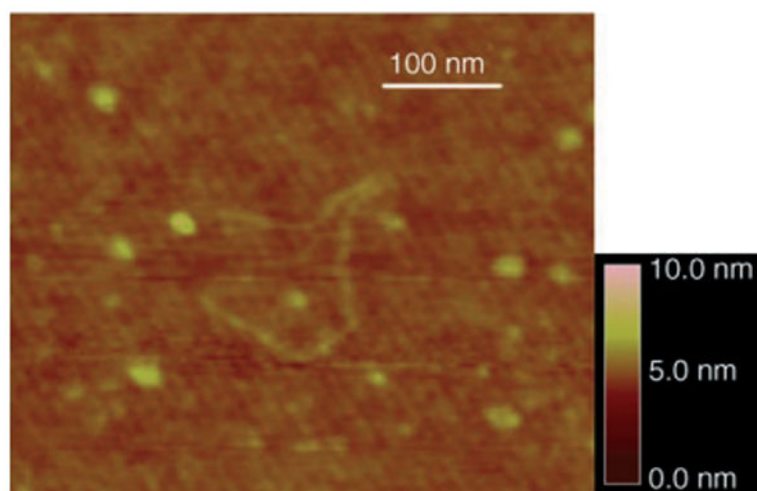
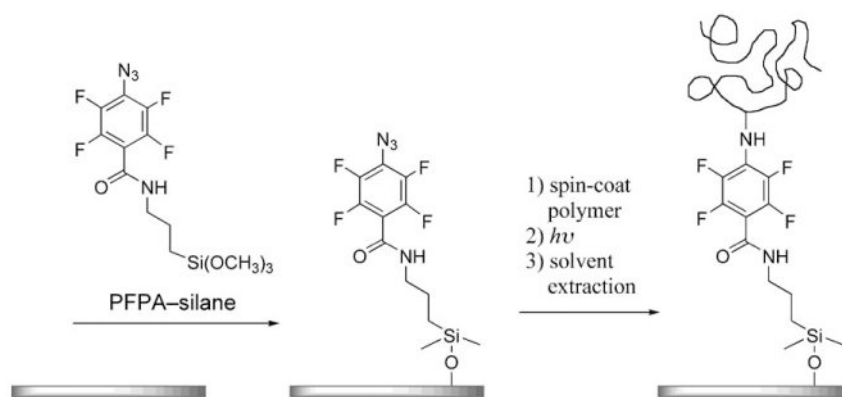


Figure 5.
An extended polystyrene molecule ($M_w = 223\,200\text{ g mol}^{-1}$).



Scheme 1.
Covalent immobilization of polymers on silicon oxide using PFPA-silane as the photo-cross-linker.

Table 1

Measured and calculated sizes of single molecules.

M_w	D [nm]	H [nm]	V [nm ³]	V_{calcd} [nm ³]
223 200	$32 \pm 6^{[a]}$	$1.6 \pm 0.6^{[a]}$	429	370
570 000	$50 \pm 7^{[b]}$	$1.9 \pm 0.5^{[b]}$	1243	947
1877 000	$69 \pm 14^{[c]}$	$2.7 \pm 1.2^{[c]}$	3364	3118

The D and H values were measured and averaged from $[a]$ 112, $[b]$ 85, and $[c]$ 51 particles from the corresponding images in Figure 2, excluding obvious large aggregates.