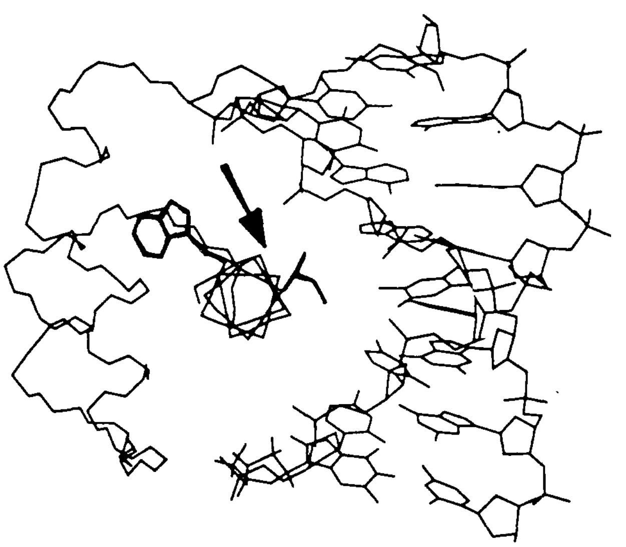

Figure 2. View along the axis of the recognition helix of one of the 16 NMR conformers representing the solution structure of the *Antp(C39S)* homeodomain–DNA complex (Billeter, M.; Qian, Y. Q.; Otting, G.; Müller, M.; Gehring, W. J.; Wüthrich, K., to be submitted). The presentation includes all heavy atoms of base pairs 5–12 of the 14-base-pair DNA, the polypeptide backbone of residues 8–56 of the *Antp(C39S)* homeodomain, and with bold lines, the side chains of Ile 47 and Trp 48 of the homeodomain. The arrow indicates the location of the hydration water molecules detected by NMR.

stringent requirements, it is likely that additional cross peaks representing intermolecular NOEs with hydration water were not identified as such. On the other hand, since several intermolecular NOEs were observed between the side chain of Ile 47 and the DNA (Billeter, M.; Qian, Y. Q.; Otting, G.; Müller, M.; Gehring, W. J.; Wüthrich, K., to be submitted), and since the amide proton of Trp 48 is next to Ile 47, we have well established that the few NOEs attributed to hydration waters are with hydrogen atoms in the interface between the protein and the DNA (Figure 2).

Only very few of the water molecules hydrating the homeodomain–DNA complex could be observed by NOEs. Apparently, most of the other solvent–solute NOEs are very weak, indicating that the residence times of water molecules in the surface hydration sites are shorter than about 0.5 ns, as was found in other peptides and proteins.[4] The negative sign and the higher intensities of the NOEs with the water molecules in the protein–DNA interface show that the lifetimes of these hydration water molecules are longer than 1 ns, and since these water molecules have the same chemical shift as the bulk water, we can also establish an upper limit for the lifetimes of about 20 ms.[12] This coincides with the behavior of hydration water in interior cavities of globular proteins, where the water molecules constitute integral parts of the molecular architecture.[4,6,12,13] The present studies thus indicate an important structural role of the hydration water in the protein–DNA interface. Since the lifetimes of these waters are much shorter than the lifetime of the complex,[14] they also show that the structure of the complex undergoes time fluctuations with similar frequencies and amplitudes as observed for globular proteins.[12]

Acknowledgment. We would like to thank Dr. W. Gehring and Dr. M. Müller, Biocenter of the University of Basel, for providing the homeodomain polypeptides used in this study, and Dr. M. Billeter for helpful discussions. Financial support was obtained from the Schweizerischer Nationalfonds (Project 31.32033.91).

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Reaction of Alkoxysilane Coupling Agents with Dehydroxylated Silica Surfaces

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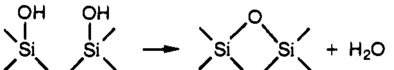
Alkoxysilane coupling agents are commonly used to bond polymers to glass surfaces by the following reaction:[1]

$$R'Si(OR)_3 + HOSi{\equiv} \rightarrow R'Si(OR)_2OSi{\equiv} + ROH \qquad (1)$$

R is typically a methyl or ethyl group, and R′ contains a functionality that can react readily with the polymer (e.g., mercaptan or epoxide). $HOSi{\equiv}$ can be either an isolated surface silanol or a hydrogen-bonded hydroxyl group. The rate of the coupling reaction can be further increased in the presence of water by first hydrolyzing the silane.[1] Until now, it was thought that reactive hydroxyl groups *must* be present on the surface in order for bonding to take place. Here we demonstrate that alkoxysilane coupling agents can react directly with highly strained siloxane bonds present on *dehydroxylated* silica, thus yielding a new mechanism for polymer–silica surface adhesion.

Strained siloxane bonds can be formed readily on high surface area silica samples by heating to temperatures in excess of 900 K.[2-4] The most reactive of these sites are edge-shared tetrahedra formed by the dehydroxylation of adjacent, isolated silanol groups.[2,3,5] The strain in these four-membered-ring structures (estimated to be 23 kcal/mol of Si–O bonds[6]) is due to the large distortion of both the O–Si–O and Si–O–Si bond angles[4,7] and results in infrared active Si–O stretching modes at 888 and 908 cm^{-1}.[2-4]



(2)

The adsorption of methyltrimethoxysilane (MTMSi) on a dehydroxylated silica surface at 330 K is followed using infrared spectroscopy (Figure 1a).[8] From this data it is clear that the intensity of the CH_3 stretching vibrations from both the methyl (asym: 2978; sym: ~2920 cm^{-1}) and methoxy (asym: 2957; sym: 2851 cm^{-1}) groups increases as a function of time.[10] A corresponding decrease in the intensity of the bands due to the Si–O stretching modes of the strained siloxane rings is also observed.[10] These data, normalized to a constant peak height, are summarized in Figure 1b.

The intensity of the isolated (i.e., non-hydrogen-bonded) O–H stretching vibration (3750 cm^{-1}) remains constant at 0.104 ± 0.001

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(8) Silica substrates were prepared using the technique described in refs 9 and 10. Approximately 12 mg of Cab-O-Sil M5 was pressed into a fine tantalum screen to make a disk ~1.2 cm in diameter and ~0.3 mm thick. Samples were then mounted in a small, turbomolecular pumped vacuum cell (base pressure of $<1 \times 10^{-8}$ Torr) and dehydroxylated for 3–6 h at $\gtrsim$1250 K by resistively heating the tantalum mesh. Spectra were collected in transmission mode using a Fourier transform infrared spectrometer (Mattson Instruments) and a liquid nitrogen cooled, wide band MCT detector. Typically 256 scans were averaged at 4 cm^{-1} resolution (~1 min acquisition time).

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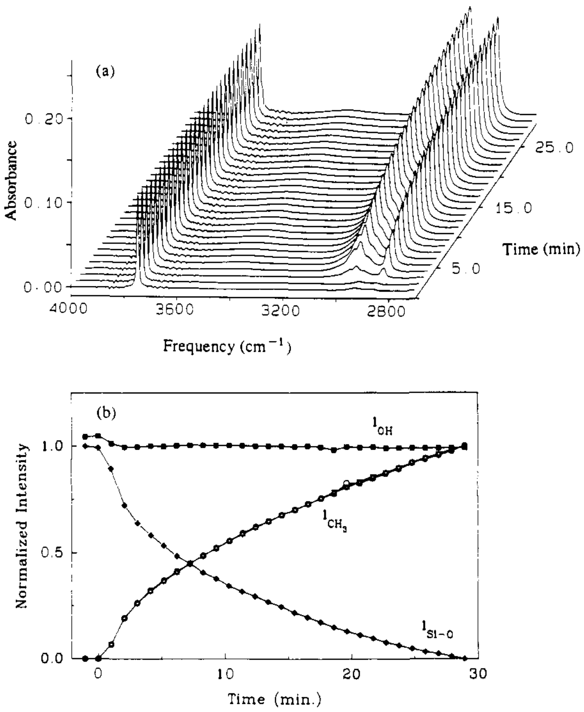
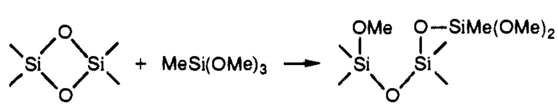


Figure 1. Infrared spectra recorded as a function of time upon exposure of a dehydroxylated silica surface to 4 mTorr of methyltrimethoxysilane at 330 K (a). Intensities of the methoxy CH$_3$ (asym: Δ, 2960 cm^{-1}; sym: ∇, 2978 cm^{-1}), SiCH$_3$ (asym: ○, 2978 cm^{-1}), Si–O (◆ ~906 cm^{-1}), and O–H (■, 3749 cm^{-1}) stretching vibrations normalized to 1 plotted as a function of time (b).

as shown by the data in Figure 1a,b. We have also found that the reactivity of the silica surface toward MTMSi *decreases* with increasing concentration of surface hydroxyl groups and *increases* with the severity of the dehydroxylation.[10] These results provide unequivocal evidence that alkoxysilanes can react directly with the highly strained rings on the dry silica surface.

The products of this reaction should be



(3)

by analogy with the reactions of water and methanol with these same highly reactive sites.[2,3,5] We expect cleavage to occur at one of the Si–O bonds of the MTMSi[11] by reaction of the basic oxygen of the adsorbate with an electron-deficient (and therefore highly Lewis acidic) surface silicon site.[5,12] This leaves two stable adsorbates: MeSi(OMe)$_2$O and MeO. The driving force for this reaction must be the release of the ring strain energy since the bonds in the resulting siloxane and alkoxide products should be the same as those in the incident MTMSi (the vibrational spectrum of the products is virtually identical to that of the gas-phase reactant[10]). No additional surface chemistry is taking place since the intensities of all of the hydrocarbon stretching vibrations increase at an identical rate (Figure 1b).

The surface alkoxides and alkylsiloxanes resulting from the reaction in eq 3 are thermally stable: the surface can be heated to 600 K with minimal loss in intensity of the infrared bands.[10] These reaction products are also stable in the presence of water vapor ($\lesssim$1 Torr) at 330 K.[10]

Finally, we have explicitly tested for the reactivity of strained siloxane bonds with several of the R′ groups found in conventional silane coupling agents. We find no significant adsorption of either mercaptans (propanethiol) or epoxides (propylene oxide) under our typical experimental conditions (4 mTorr gas pressure, 330 K sample temperature, 30 min).[13] Thus, these groups are still free to bind into the polymer network.

In conclusion, this work provides a new mechanism by which silane coupling agents can bond to silica surfaces: the nucleophilic attack of an alkoxysilane on a highly strained siloxane ring of the substrate.[14] The extent of this reaction scales *inversely* with the surface hydroxyl concentration and *increases* with the dehydroxylation temperature (and therefore the number of strained siloxane bonds), explicitly showing that surface hydroxyl groups are *not* involved in the reaction. The use of this novel concept to actually improve polymer–surface adhesion remains to be exploited.

Acknowledgment. We thank E. A. Chandross for several helpful discussions and R. W. Filas for the loan of many of the alkoxysilanes used in this study.

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(12) The Lewis basic surface oxygen atoms may also be playing a role in the reaction, although it is expected to be small.[5] In either case, the products are the same.

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(14) The dissociative adsorption of alkoxysilanes is *not* limited to trifunctional adsorbates, as we have observed a similar series of vibrational spectra after exposing a clean, dehydroxylated silica surface to 4 mTorr of bifunctional dimethyldimethoxysilane and methyldimethoxysilane and monofunctional trimethylmethoxysilane at 330 K. Ethoxysilanes (including methyltriethoxysilane and trimethylethoxysilane) and silanols (triethylsilanol) are also effective at cleaving strained Si–O–Si bonds. All of these adsorbates have thermal and hydrolytic stability similar to that of MTMSi.[10]

Effect of Adsorbate Coverage and Ion Beam Exposure Time on Reactive Ion–Surface Collisions: $C_5H_5N^+$ Reactions with $C_5D_5N/Ag(111)$

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Much experimental effort has been applied to the study of the scattered ions which are produced in molecular ion–surface collisions.[1-12] These experiments have been developing surface-induced dissociation as a practical method of fragmenting organic ions in tandem mass spectrometry and have sought to understand on a fundamental level both dissociative and reactive ion–surface collisions. However, most experiments studying molecular ion–surface interactions have been limited by the lack of well-characterized surfaces and/or the ability to control in situ the adsorbates under ultrahigh vacuum (UHV) conditions. By application of UHV surface science methods to the study of reactive ion–surface collisions for the pyridine/Ag(111) system, it is shown here that scattered ion yields can be strongly dependent

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