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Formation and *in Situ* Modification of Monolayers Chemisorbed on Ultraflat Template-Stripped Gold Surfaces

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We have previously described the preparation of ultraflat Au(111) surfaces as substrates for scanning probe microscopy. We report here alternative ways to produce polycrystalline Au(111) thin films of similar, high quality (i.e., with mean roughness smaller than 5 Å over 25 μm^2). All of them are based on the same principle, i.e., that of exposing the very first layer of gold atoms which had deposited onto mica. One alternative route leads to substrates which are transparent enough for optical microscopy. Two other routes make use of ceramic glues, providing substrates which can be handled with most organic solvents without disruption of the Au(111) layer. The substrates prepared by the latter procedures can thus be used to produce gold-directed self-assembled monolayers (SAMs) from nearly all ω-functionalized alkanethiols or dialkyl disulfides; they can also be used for the *in situ* chemical modification of SAMs on gold. For this purpose, appropriate reaction chambers have been developed. As an example of the use of the new techniques described here, we report the preparation of a SAM of *N*-palmitoylcysteamine, either from *ex situ* synthesis of the precursor or from *in situ* acylation of the amino head groups of a cysteamine SAM.

Introduction

Scanning tunneling microscopy and atomic force microscopy are now well-established analytical methods in solid state physics and materials science. They are also becoming more and more important tools in structural research in biology and organic chemistry, as demonstrated by the increased number of publications in these fields which have appeared in the last few years.[1−6] However, scanning probe microscopy (SPM, as these and related techniques are collectively designated) has not yet become routine with sensitive samples such as biomolecules or organic structures; *i.e.*, the research in this field is still focused on several methodological aspects.

Chemical and physical properties of the substrate are of primary importance. In addition to being chemically well defined and inert against O_2, yet endowed with specific chemical reactivity, the substrate must be atomically flat over large areas; if not, objects having unfavorable length: width ratios will "sink" into an irregular topography. This is especially true for fibrillary structures of μm in length, *e.g.*, nucleic acids or organic polymers with diameters smaller than 3 nm, but holds true also for individual globular proteins. These macromolecules require substrates with a mean roughness as small as a few angstroms on areas of at least 5 μm^2.

Substrates of very low roughness are advantageous also for the formation of self-assembled monolayers (SAMs) and in the investigation of phenomena such as adsorption, adhesion, wetting, lubrication, or friction. Finally, the ideal substrates should also be reasonably priced and easy to prepare, to store, and to chemically modify.

Gold is becoming a popular substrate. For most purposes its surface is satisfactorily inert, but can easily chemisorb dialkyl disulfides or alkanethiols, which spontaneously organize into regular SAMs.[7−11] In addition, if ω-functionalized, these monolayers should in principle allow a variety of novel and sophisticated molecular architectures. Gold surfaces thus have the potential of being important tools in the expanding field of SAMs for chemical sensing, nonlinear optics, and lithography.[12]

Several procedures have been reported which yield atomically flat gold terraces over several 100 nm.[13−16] The quality of these surfaces, (*i.e.*, flatness over large areas) generally lags behind that of mica, however. With the ultimate goal of preparing ω-functionalized bioreactive SAMs on gold in order to image immobilized biological structures by SPM, we have recently developed a procedure to prepare Au(111) surfaces with a mean roughness of 2−5 Å on areas of at least 25 μm^2.[17] Briefly (see Figure 1A): gold is first grown epitaxially onto mica (which is among the flattest surfaces known); the gold layer is then glued onto a silicon wafer and the mica is stripped off. The uncovered, template-stripped gold surface (TSG) (*i.e.*, the very first atom layer of gold having deposited onto the mica) is nearly as flat as mica itself but has the favorable chemical properties of gold. On these ultraflat, polycrys-

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(1) Yang, J.; Tamm, L. K.; Somlyo, A. P.; Shao, Z. *J. Microsc. (Oxford)* **1993**, *171*, 183.

(2) Engel, A. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 79.

(3) Frommer, J. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1298.

(4) Hansma, H. G.; Hoh, J. H. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 115.

(5) Lal, R.; John, S. A. *Am. J. Physiol.* **1994**, *266*, C1.

(6) Morris, V. J. *Prog. Biophys. Molec. Biol.* **1994**, *61*, 131.

(7) Sellers, H.; Ulman, A.; Shnidman, Y.; Eilers, J. E. *J. Am. Chem. Soc.* **1993**, *115*, 9389.

(8) Ulman A. *An Introduction to Ultrathin Organic Films From Langmuir−Blodgett to Self-Assembly*; Academic Press, Inc.: San Diego, CA, 1991.

(9) Whitesides, G. M.; Laibinis, P. E. *Langmuir* **1990**, *6*, 87.

(10) Bain, C. D.; Troughton, E. B.; Tao, Y.-T.; Evall, J.; Whitesides, G. M.; Nuzzo, R. G. *J. Am. Chem. Soc.* **1989**, *111*, 321.

(11) Nuzzo, R. G.; Allara, D. L. *J. Am. Chem. Soc.* **1983**, *105*, 4481.

(12) Kumar, A.; Whitesides, G. M. *Appl. Phys. Lett.* **1993**, *63*, 2002.

(13) Clemmer, C. R.; Beebe, T. P. *Scanning Microscopy* **1992**, *6*, 319.

(14) Chidsey, C. E. D.; Loiacono, D. N.; Sleator, T.; Nakahara, S. *Surf. Sci.* **1988**, *200*, 45.

(15) Putnam, A.; Blackford, B. L.; Jericho, M. H.; Watanabe, M. O. *Surf. Sci.* **1989**, *217*, 276.

(16) DeRose, J. A.; Thundat, T.; Nagahara, L. A.; Lindsay, S. M. *Surf. Sci.* **1991**, *256*, 102.

(17) Hegner, M.; Wagner, P.; Semenza, G. *Surf. Sci.* **1993**, *291*, 39.

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talline Au(111) surfaces, SPM measurements of monolayers or of biological materials chemisorbed via thiolate bonds are possible even in aqueous buffers or other fluids. Our procedure should not be confused with techniques which have proved their value in electron microscopy, in which, *e.g.*, biological objects are first deposited onto mica, embedded in platinum or graphite, and eventually imaged in them after removal of mica.[18,19]

The epoxy-glued TSGs we have previously described are excellent for use in aqueous environments or in those organic solvents which do not adversely affect the organic glue, *i.e.*, ethanol, acetone, dioxane, etc.[17] Thus, this type of TSG is recommended for preparing SAMs from ethanol-soluble compounds, *e.g.*, alkanethiols, or for immobilizing biological structures carrying accessible thiols. However, epoxy-glued TSGs are not suitable as an interface during chemical derivatization of the substrate or the adsorbed monolayers, or in the handling of functionalized long-chain thiols or disulfides, if solvents such as tetrahydrofuran or chloroform are required. These solvents cause the epoxy glue to swell excessively, thereby leading to disruption of the overlaying gold film.

In the following text, we first describe new applications and alternative ways of preparing TSGs (see Figure 1B–D). Among the novel Au(111)-directed SAMs, we describe here an optically transparent epoxy-glued template-stripped gold surface, which enables investigations on hybrid atomic force light microscopes and combines the possibility of preparing functionalized, ultraflat surfaces with optical transparency. Binding of biological objects by SAMs on glass-supported gold films has been reported very recently in ref 20 also.

As an alternative to the epoxy glues used by us to bind the gold layer to its final support we have now investigated ceramic glues. These inorganic glues are insensitive to organic solvents and to the harsh conditions which may be required for the chemical modification of monolayers chemisorbed to the Au(111) surfaces.

We have also developed a very flexible laboratory setup which allows preparation, modification, and wet chemical analysis of SAMs on TSG. As an example, we describe here the preparation and characterization of a SAM of *N*-palmitoylcysteamine onto these Au(111) surfaces; these monolayers were prepared by either of two ways: by chemisorption of presynthesized *N,N'*-dipalmitoylcystamine onto ceramic-glued TSG or else by first preparing a SAM of cysteamine onto epoxy-glued TSG and then reacting the amino groups of the chemisorbed cysteamine with palmitoyl *N*-hydroxysuccinimide ester.

Experimental Section

Instrumentation and Materials. Gold (99.99%) was purchased from Cendres & Metaux SA (Biel, Switzerland) and ruby muscovite mica from Bal-Tec (Balzers, Liechtenstein). Monocrystalline Si(100) wafers were from Faselec (Zurich, Switzerland). The epoxy glues Epo-tek 377 and 301-2 were obtained from Polyscience (Zug, Switzerland), the ceramic glue Cerastil C7 from Wahl & Lehmann (Urdorf, Switzerland), and Panavia 21 from Cavex (Haarlem, Holland). The adhesive tape was a commonly available Scotch Tape (3M). All chemicals and solvents were commercial grades of highest purity.

A vacuum coating system BAE 370 from Bal-Tec (Balzers, Liechtenstein) with thermal evaporation system BSV 203 (Balzers, Liechtenstein), electron beam gun (Telemark, San Jose, CA), integrated quartz crystal deposition controller Inficon XTC/2 from Leybold (Zurich, Switzerland), and specially designed substrate heater was used to deposit metal films on mica. Scanning tunneling microscopy was carried out either on a Nanoscope III from Digital Instruments, Inc. (Santa Barbara, CA) with a modified STM tip-view head for high tunneling resistance limit of 100 GΩ) or on a home-built STM with tunneling resistance in the TΩ-range. ^{1}H-NMR spectra were recorded on Bruker instruments. Chemical shifts (δ) are reported in ppm relative to internal standard (($(CH_3)_4Si$, $\delta = 0.00$). A Plasmos SD 2300 ellipsometer (Polyscience, Zug, Switzerland) was used for thickness measurements using a 70° angle of incidence with an ~1 mm diameter beam at 623.8 nm (He–Ne laser). All melting points (mp) are uncorrected.

Syntheses (See Also Figure 5). *Hexadecanoic Acid 2,5-Dioxopyrrolidin-1-yl Ester* (**2**) *(Palmitoyl N-Hydroxysuccinimide Ester).* To a solution of palmitic acid (**1**) (15.4 g, 60 mmol) in DMF (100 mL) was added *N*-hydroxysuccinimide (HOSu) (6.9 g, 60 mmol) followed by DCC (13.6 g, 60 mmol). After the solution was stirred for 36 h at room temperature, the dicyclohexylurea (DCU) was filtered. Removal of the solvent under reduced pressure and recrystallization from 2-propanol provided **2** as a white solid (18.9 g, 89%): mp 86 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.83 (s, 4H, $C(O)CH_2CH_2C(O)$), 2.60 (t, 2H, CH_2COO, $J = 7.5$ Hz), 1.74 (m, 2H), 1.26 (m, 24H), and 0.88 (t, 3H, $J = 6.7$ Hz). Anal. Calcd for $C_{20}H_{35}NO_4$: C, 67.95; H, 9.98; N, 3.96. Found: C, 68.09; H, 9.77; N, 3.94.

N,N'-(Dithiodi-1,2-ethanediyl)dihexadecanamide **4** *(N,N'-Dipalmitoylcystamine).* Compound **2** (4.57 g, 13 mmol) was dissolved in THF (60 mL), and then a solution of cystamine dihydrochloride **3** (1.46 g, 6.5 mmol) and $NaHCO_3$ (2.18 g, 26 mmol) in 50 mL of water and 70 mL of THF was added over a period of 2 h at 0 °C. After 40 min, a white solid started to precipitate. The reaction mixture was allowed to warm to 23 °C and was stirred for 15 h until TLC analysis showed complete reaction. The THF was removed under reduced pressure and the residue acidified with citric acid to pH 3. The aqueous solution was extracted with chloroform, and the organic phase was then washed three times with water, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Recrystallization from hexane–chloroform gave **4** as a white solid (3.89 g, 95%): mp 123 °C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 95:5) δ 3.51 (t, 2H, $J = 6.6$ Hz), 2.82 (t, 2H, $J = 6.6$ Hz), 2.20 (t, 2H, $J = 7.6$ Hz), 1.26 –1.63 (m, 26H), and 0.88 (t, 3H, $J = 6.9$ Hz). Anal. Calcd for $C_{36}H_{72}N_2O_2S_2$: C, 68.73; H, 11.54; N, 4.45; S, 10.19. Found: C, 69.06; H, 11.51; N, 4.47; S, 10.22.

Preparation of Template-Stripped Gold Surfaces (TSGs). *TSG Based on Epo-tek 377 (Figure 1A).* Freshly cleaved ruby muscovite mica sheets were placed cleaved side down into the molybdenum sample holder of our vacuum system BAE 370. The mica sheet, which typically had a size of 35 cm^2, was radiatively heated *in situ* from the rear through the sample holder. Temperature feedback control was carried out with a chromel–alumel thermocouple. Before the evaporation of gold was started, the mica sheet was heated for more than 12 h at 300 °C at a pressure of $< 1.33 \times 10^{-4}$ Pa (10^{-6} Torr) to outgas adsorbed water and other volatile contaminants. Gold films, 200 nm thick, were vapor-deposited at 300 °C either in one (Figure 2E,F) or in two steps (Figure 2B,C) by first depositing a 5 nm thick layer, annealing for 6 h at the same temperature, and finally depositing a second, 195 nm thick layer. Evaporation rates were typically 1 Å/s. As described previously,[17] the gold-deposited mica sheets were cut into pieces (~1 cm^2) and glued gold face down onto Si(100) wafer pieces or glass coverslips using 10 μL of Epo-tek 377 (a particular epoxy glue of amber color which is often used in laser and fibre optics). Epo-tek 377 consists of two components (resin and hardener). It is essential to thoroughly mix the two components in equal parts (by weight)! Curing was finished by heating the multilayer at 150 °C for 1–2 h. The resulting Si–epoxy glue–Au–mica "epoxy-sandwiches" could be stored as "stripping precursors" at least up to several months without detectable loss of quality. Before use, these stripping precursors were soaked in THF at room temperature, which leads to complete detachment of the mica layer, thereby exposing the gold film ("chemically stripped gold surface"). One could help the detachment of the mica from the gold layer by exerting a slight suction onto the mica sheet (e.g., using a plastic tubing connected to vacuum).[21] Immediately thereafter, the chemically stripped fresh gold surfaces were washed with THF, checked for conductivity,

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(18) Butt, H. J.; Müller, T.; Gross, H. *J. Struct. Biol.* **1993**, *110*, 127.

(19) Blackford, B. L.; Jericho, M. H. *J. Vac. Sci. Technol. B* **1991**, *9*, 1253.

(20) DiMilla, P. A.; Folkers, J. P.; Biebuyck, H. A.; Härter, R.; López, G. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1994**, *116*, 2225.

and used for monolayer preparation. Figure 2B−D shows STM images of such chemically-stripped gold surfaces.

TSG Based on Epo-tek 301-2 (Figure 1B). For transparent gold surfaces, 20 nm of gold was deposited on mica at a substrate temperature of 300 °C, followed by annealing for 2 h. The gold-deposited mica sheets were cut and glued gold face down on glass coverslips with $10\ \mu L/cm^2$ Epo-tek 301-2 (which is colorless and transparent; resin and hardener have to be thoroughly mixed (100:35) by weight!) as described above. Curing was finished by heating the multilayer at 80 °C for 3 h. The mica can be removed as described for Epo-tek 377 sandwiches.

TSG Based on Panavia 21 (Figure 1C). Gluing with Panavia 21 required an additional 200 nm thick layer of tin onto the 200 nm gold film as adhesion mediator; it could be deposited immediately after the gold by thermal evaporation at room temperature. After the Sn−Au−mica multilayer was cut, the pieces were covered with approximately 50 μL of Panavia 21 (freshly mixed both components from a dispenser system) and glued tin face down onto a support (steps 5a or 5b in Figure 1C). After 30 min curing at room temperature under exclusion of oxygen (i.e., in an argon atmosphere), the residual unpolymerized glue at the borders was removed by washing in ethanol. The mica (with typical thickness of about 40 μm) was then carefully stripped off in successive layers using an adhesive (Scotch) tape ("mechanically stripped gold surface") (step 6 in Figure 1C). After each stripping the conductivity of the surface was checked and finally the surface examined by light microscopy. This mechanical procedure requires some practical experience, and its success depends often on the quality of the mica used. The few gold films (less than 5%) with residues of mica islands were discarded. As supports we used either rough surfaces (e.g., metals or ceramic platelets) or very smooth supports (e.g., the polished side of a silicon wafer). The latter exhibited a low adhesion to the Panavia glue; the wafer platelet could thus be removed from the multilayer alternatively by soaking in tetrahydrofuran for a couple of minutes (step 7 in Figure 1C). The resulting Panavia glue−Sn−Au triple layer is rigid enough and can be mounted without additional support in a scanning probe microscope.

TSG Based on Cerastil C7 (Figure 1D). In order to use Cerastil C7 to glue 200 nm Au surfaces resulting from step 2, a 10 nm titanium layer followed by another 150 nm thick silicon dioxide layer was deposited by electron-beam evaporation in the same deposition chamber at room temperature. The SiO_2−Ti−Au−mica multilayer was cut and glued with a creamy suspension of Cerastil C7 (1 mm thickness) onto Si(100) wafer pieces (we recommend to thoroughly grind the cement). After being cured for 24 h at room temperature and then for 2 h at 70 °C, the Si/SiO_2−ceramic glue−SiO_2−Ti−Au−mica multilayer "ceramic sandwiches" were extensively rinsed in water (18 MΩcm) at 70 °C; the mica was removed with an adhesive tape as described above (Figure 1D, step 7).

Formation of SAMs (Figure 5).[22] SAM **4c** was prepared on TSG films glued with Cerastil C7 which were placed in the reaction chamber first (Figure 4, referred to in detail in the Results and Discussion). Five mL of a 1 mM solution of **4** in chloroform−methanol (95:5, v/v) was added. After incubation at 50 °C for 12 h, the solution was removed and the monolayer rinsed with chloroform−methanol at 50 °C and dried under a stream of nitrogen.

SAM **3e** was similarly prepared on epoxy-glued (Epo-tek 377) TSG films (also mounted in the reaction chamber) using 5 mL of a 1 mM solution of cysteamine dihydrochloride **3** in water. After 12 h at room temperature, the film was washed repeatedly with 3 mL of 1 M NaOH and then with water (18 MΩcm) and finally dried under a stream of nitrogen. For preparation of SAM **4e**, platelets with freshly formed SAM **3e** were exposed to a solution of compound **2** (10 mM in acetone) for 12 h at room temperature, then extensively rinsed first with acetone and then with water (the disappearance of **2** was followed by TLC analysis), and dried under a stream of nitrogen.

Monolayers of 1-dodecanethiol on Au(111) were also prepared in the reaction chamber using a solution of 1-dodecanethiol (1 mM in ethanol) for 2 h at room temperature, followed by thorough rinsing with ethanol and drying in vacuum.[23]

STM Measurements. All STM data were acquired with Pt/Ir (90:10) tips, which were either mechanically prepared (cut) or made by electroetching of a 0.25 mm wire in 2.6 M KCl, 0.4 N HCl, at an AC voltage of 12 V down to 2 V. Tunneling currents (I_{tun}) were typically 0.5 nA and tip bias voltages (V_{bias}) were −800 mV for large scans or 2−6 nA and −5 to −10 mV for atomic resolution on bare gold surfaces (tip negative with respect to the sample). Tunneling conditions with resistances up to 1 TΩ were used for nondestructive imaging of self-assembled monolayers. All STM images were taken in the constant-current imaging mode. The lattice constants were measured on Au(111) and on HOPG, and the known parameters were obtained; *z*-calibration was confirmed by measurement of Au(111) step heights. All images shown in this paper are from unfiltered data.

Results and Discussion

TSG Surfaces: Preparation Procedures. TSG as a gold substrate for scanning probe microscopy is particularly suitable in biological investigations.[24,25] Preparing TSG requires fixing the thin gold film onto a support providing adequate mechanical stability once the mica sheet will have been removed. We normally did this by gluing the gold side onto a platelet of silicon or glass (Figure 1A,B). The general applicability of this method is limited, however, by the physical and chemical stability of the glue. This is important when either complex chemical *in situ* modifications may be required or with compounds which may need appropriate solvents for their assembly as monolayers. In order to overcome this problem we have screened dozens of commercially available glues, either alone, or in combination with metal films or silicon dioxide as interlayers (needed with some glues for adequate adhesion).

As a result, we present here four routes to prepare TSGs which should meet most requirements we can now think of (Table 1 compares the glues used in this paper). The schematic diagrams (not to scale) in Figure 1A,B demonstrate two methods based on epoxy-glued supports: at left (A) the procedure which we have described previously[17] and at right (B) a variation to prepare transparent TSGs for applications requiring samples which transmit visible light. Optical transparency offers the possibility to study molecules, organelles, or cells covalently immobilized on ultraflat gold with phase contrast microscopes, transmission spectroscopy, or hybrid optical atomic force microscopes.[26] Thus, epoxy-glued TSGs are prepared either with Epo-tek 377 (Figure 1A) or with Epo-tek 301-2 (Figure 1B) (other epoxy glues have proved unsatisfactory). The glues chosen have several advantages: shorter deposition procedures and chemical stripping by short incubation in THF due, we believe, to limited swelling. Further, these

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(21) The mechanism whereby THF leads to the detachment of the gold from the mica is not known. Probably, THF produces a slight expansion of the epoxy glue, which is followed by the ductile gold layer but not from the stiff mica.

(22) Strictly speaking, the term self-assembled monolayer refers to the spontaneous association of organosulfur compounds driven by the energies associated with chemisorption and interchain van der Waals interactions which could indeed be similar in size (see also: Sellers, H.; Ulman, A.; Shnidman, Y.; Eilers, J. E. *J. Am. Chem. Soc.* **1993**, 115, 9389). The latter ones are proportional to the chain lengths, and the adsorption layer **3e** made of the short compound **3** should thus not be described as a tightly packed SAM. But due to the fact that short alkanethiols such as ethanethiol show also $(\sqrt{3} \times \sqrt{3})R30^\circ$ adlayers on Au(111) lattices (see ref 37) and **3e** exhibits complete coverage with an ellipsometrical thickness of approximately 4 Å, we use this term also for **3e**.

(23) For this type of SAM, the use of the reaction chamber is not necessary, since the epoxy glue is absolutely stable in ethanol and one can immerse the complete "epoxy-sandwich" into the thiol solution.

(24) Hegner, M.; Wagner, P.; Semenza, G. *FEBS Lett.* **1993**, *336*, 452.

(25) Wagner, P.; Kernen, P.; Hegner, M.; Ungewickell, E.; Semenza, G. *FEBS Lett.* **1994**, *356*, 267.

(26) The 20 nm thin, transparent TSG surfaces are blue in color and have less electrical conductivity than 200 nm thick gold films. The conductivity decreases with increasing annealing times and temperatures; this is a matter for future studies.

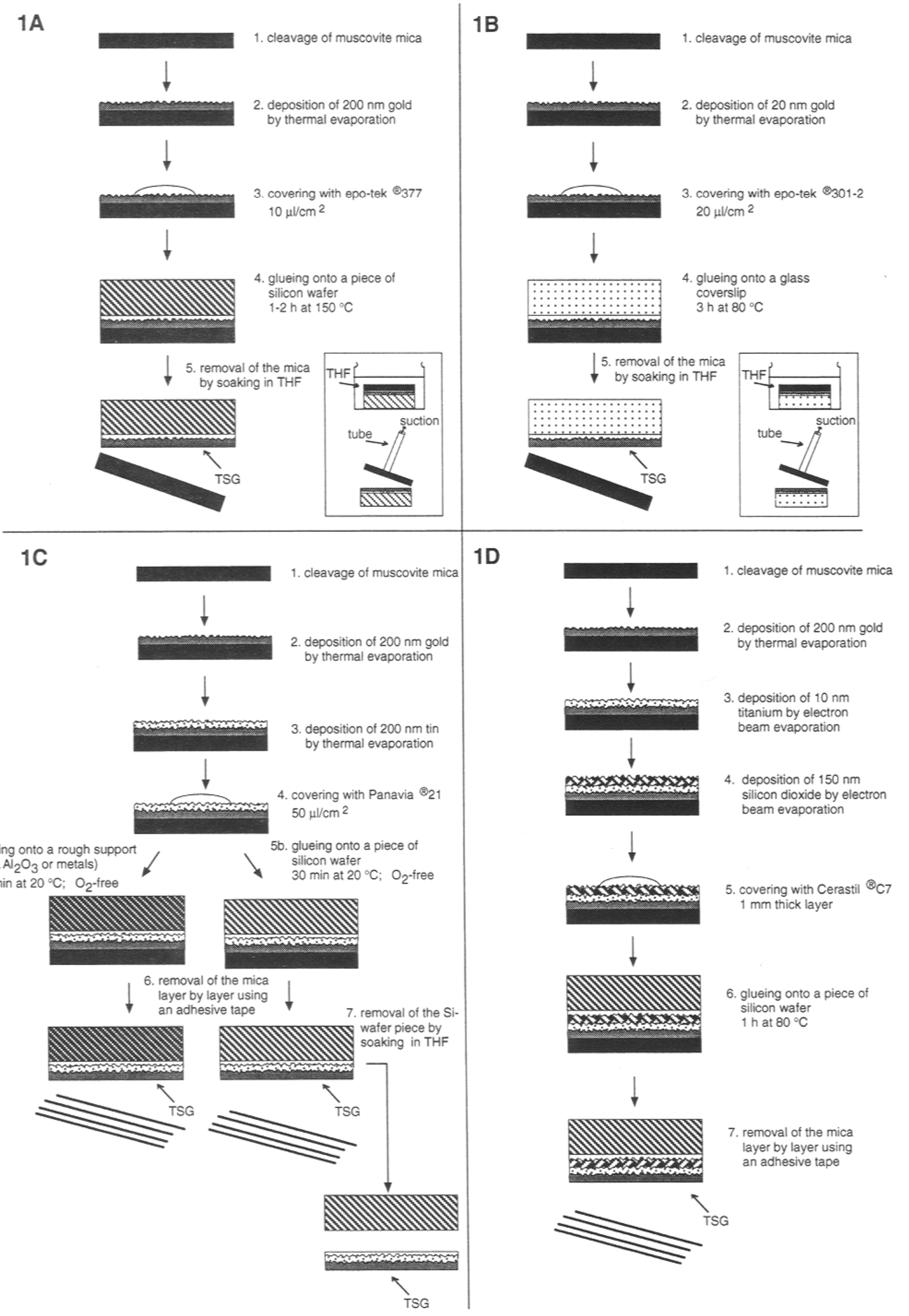

Figure 1. Preparation of template-stripped gold surfaces: (A) procedure using Epo-tek 377 as previously described;[17] (B) procedure using Epo-tek 301-2 to prepare transparent TSGs; (C) procedure using Panavia 21 and tin adhesion mediator; (D) procedure using Cerastil C7 and Ti/SiO_2 adhesion mediators.

Table 1. Comparison of the Four Main Glues Chosen for the Present Work

glue	applications	advantages	disadvantages
Epo-tek 377	use in water, buffer solutions, some organic solvents (e.g., ethanol, methanol, dioxane, acetone)	• easy to use • chemical stripping by soaking in THF • inexpensive	• limited stability in organic solvents
Epo-tek 301-2	see Epo-tek 377	• colorless • easy to use • chemical stripping by soaking in THF • inexpensive	• limited stability in organic solvents
Panavia 21	systems with organic and aqueous solvents	• high stability in organic solvents • easy to use • fast (dispenser system)	• mechanical stripping layer by layer • fairly expensive
Cerastil C7	systems with organic solvents	• high stability in organic solvents • inexpensive	• grain size • mechanical stripping layer by layer

epoxy glues are stable in alcohols, in 1,4-dioxane, and in aqueous environments and reasonably stable for several hours in acetone; they are thus recommended for most biological purposes and when using thiols or disulfides which are soluble in those solvents. On the other hand, longer exposure to other solvents such as chloroform, dichloromethane, or THF leads to complete disruption of the gold films due to excessive swelling of the underlaying epoxy glues.

In contrast, the "ceramic-sandwiches", which we report here for the first time (Figure 1C,D), are inert against organic solvents and moderate acids or bases. They are based on two commercially available ceramic glues: Panavia 21 and Cerastil C7. The first is used as a dental adhesive and consists of 10-methacryloyldecyl dihydrogen phosphate (MDP) as the monomer and 77% inorganic filler (silanated silica and barium glass powder). The second is a heat-resistant ceramic filler for electric components and is based on potassium silicate.

With both ceramic glues, one has to accept somewhat longer preparation times. In fact, all resin cements tested exhibit low adhesion on gold surfaces and thus require deposition of additional thin interlayers of either tin (Figure 1C) or titanium/silicon dioxide (Figure 1D).[27] Routinely, we deposited by thermal evaporation a 200 nm thin tin layer (Figure 1C) or by electron beam evaporation a 10 nm thin titanium film plus 150 nm thick silicon dioxide film as the adhesion mediators to gold (Figure 1D). A disadvantage of the multilayer plus ceramic glue (Figure 1C,D) is that it is impossible to remove the mica by chemical stripping, which mandates mechanical stripping with an adhesive tape. This step needs some practical experience. A second stripping step could be carried out as shown in Figure 1C, step 7: If glued on a very smooth surface (e.g., onto the polished side of a silicon wafer), the Sn-mediated TSG surface can be supported by the polymerized Panavia 21 cement alone. This could be achieved by soaking the whole multilayer in THF, which leads to the separation of the Panavia glue−Sn−Au stack from the Si wafer; i.e., there is no need to use additional supports for the 400 nm thick Sn/Au layer.

TSG Surfaces: STM Analyses. Prior to stripping, the multilayers can be stored as precursors for months, indeed for unlimited time, as we detected no deterioration in the quality of the Au(111) surfaces even after 1 year. This is clearly important for routine work, when hundreds of samples are to be examined each month.

As judged by STM, epoxy- and ceramic-glued TSG surfaces are of equally high quality and are vastly superior to the upper surfaces of epitaxially grown gold. This is particularly important in the investigation of large objects, when very small roughness over large areas is a must. Figure 2 compares such an upper surface of gold which was deposited on mica at 300 °C (Figure 2A) with epoxy-glued template-stripped surfaces (Figure 2B−D) and ceramic-glued template-stripped surfaces (Figure 2E,F) which originally faced the mica. The STM image in Figure 2A captured with a z-range of 50 nm shows the usual topography of an upper Au surface. Atomically flat

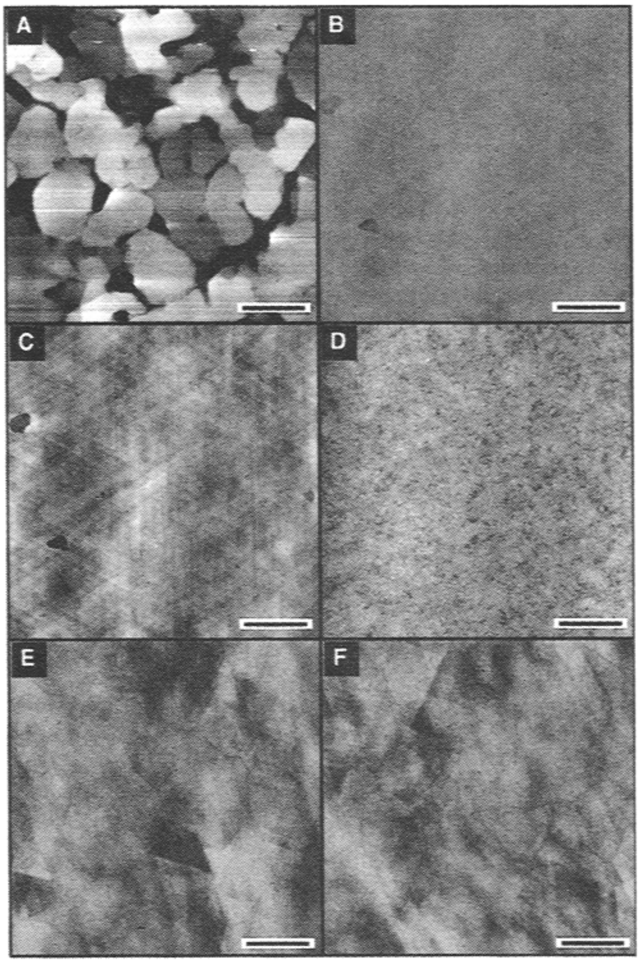

Figure 2. STM images of epitaxially grown Au(111) films on mica (bars = 1 μm). Tunnel parameters: $V_t = 0.2$ V and $I_t = 1$ nA. Key: (A) conventional Au(111) surface deposited on mica at 300 °C (z-range = 50 nm; mean roughness = 32.5 Å); (B) template-stripped gold (TSG) surface deposited in two steps on mica at 300 °C and prepared with Epo-tek 377 (z-range = 50 nm; mean roughness = 2.6 Å); (C) same as in (B), but z-range = 4 nm; (D) TSG surface deposited on mica at 20 °C and prepared with Epo-tek 377 (z-range = 4 nm; mean roughness = 2.2 Å); (E) TSG surface deposited on mica at 300 °C and prepared with Cerastil C7 (z-range 3 nm; mean roughness = 2.3 Å); (F) TSG surface deposited on mica at 300 °C and prepared with Panavia 21 (z-range 3 nm; mean roughness = 3.1 Å).

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[27] A similar, established technique makes use of chromium or titanium interlayers for vapor-deposited gold films on silicon substrates; see e.g.: Abbott, N. L.; Rolison, D. R.; Whitesides, G. M. *Langmuir* **1994**, *10*, 2672.

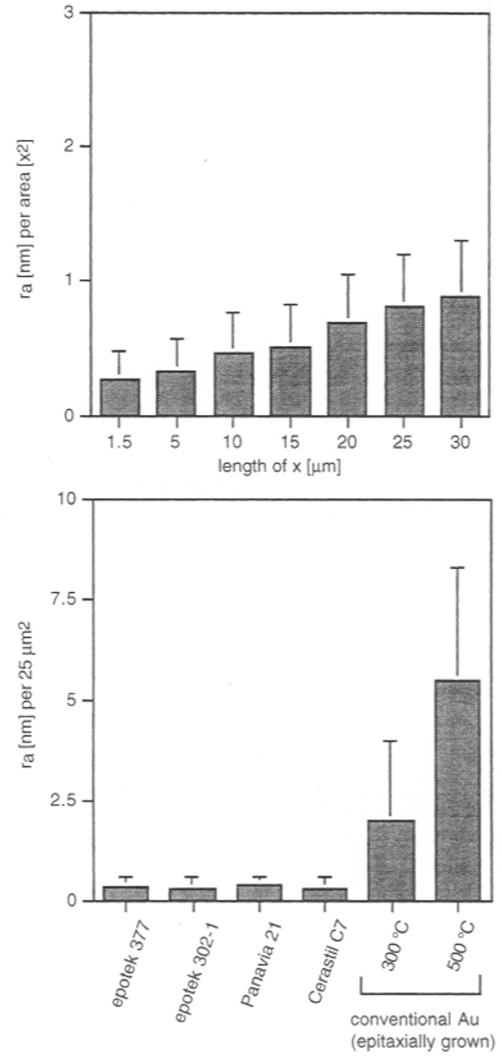

Figure 3. (Top) mean roughness of TSG surfaces on different lengths, glued on Epo-tek 377. (Bottom) mean roughness of different types of TSGs and of conventional epitaxially grown Au(111) surfaces on mica.

terraces seldom larger than approximately 1 μm in size are separated by deep grooves and holes with extremely large differences in z-heights. These structural features are quantitated by the observed excessively large mean roughness values measured over a μm range; they may also reduce the packing of self-assembled alkanethiolates and make SPM imaging of fibrillary structures difficult. In contrast, the TSG surface of Figure 2B,C (deposited in two steps at a substrate temperature of 300 °C and glued on Epo-tek 377) was captured with the same z-contrast (50 nm) and exhibited a completely annealed surface, comprising terraces of as few as three to five atomic steps. The same image is shown in Figure 2C with enhanced contrast (z-range 4 nm). Thus, the mean roughness of TSG is at least 10 times better (*i.e.*, 2–5 Å) than conventional gold surfaces (see also Figure 3). The ceramic-glued TSGs shown in Figure 2E,F have a comparable flatness (see also Figure 3) of 2.28 Å over 25 μm^2 (Cerastil C7) or 3.10 Å over 25 μm^2 (Panavia 21), respectively. The substrate temperature during high vacuum deposition is less critical than for the commonly used top surface, where coalescence usually starts at 300 °C (which is necessary for the formation of atomically flat terraces).

Typical triangular facets are seen on TSG, if deposited at a substrate temperature of 300 °C. These facets are especially more frequent if deposited in two steps by first depositing a 5 nm thick layer, followed by annealing for 6 h at the same temperature, and finally depositing a second, 195 nm thick layer (Figure 2B,C), compared to a 200 nm one-step deposition (Figure 2E,F).[28] Occasionally, holes with diameters <200 nm are visible, arising during the deposition of gold or during the stripping step.

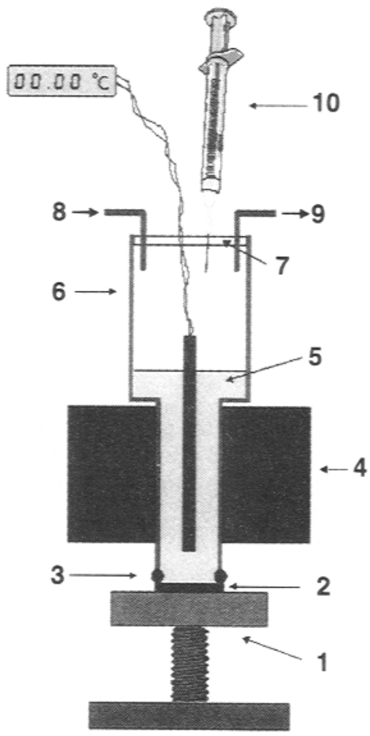

Figure 4. Reaction chamber for *in situ* synthesis, analyses, and modification of SAMs on TSG (1, PTFE base; 2, template-stripped gold film; 3, KALREZ O-ring; 4, heating/cooling coat; 5, reaction medium with Pt100 thermosensor; 6, reaction chamber (glass); 7, silicon seal; 8, inert gas inlet; 9, inert gas outlet; 10, syringe for reagents).

The TSG surface of Figure 2D was deposited on preheated mica at room temperature, which resulted in surfaces with similar flatness, i.e., crystallites with the same z-heights, but exhibiting a flat topography with fewer triangular facets and with very small polycrystalline grains.

STM images with atomic resolution are routinely achieved from these TSG surfaces (data not shown) showing Au(111) orientation with a lattice spacing of 2.88 Å. X-ray photoelectron spectroscopy (XPS) analyses showed that only trace amounts of Si and Al from the original mica were left on the Au(111) surface (data not shown). We are presently working out the conditions to produce template-stripped flat surfaces of silver and platinum also.

A Reaction Chamber. As an additional step toward expanding the potentialities of a better controlled use of TSGs, we have developed a multipurpose chamber for *in situ* reactions. It exposes to reagents the gold surface only and, thus, renders possible the chemical modification of Au or of the monolayers adsorbed thereon. The setup is shown in Figure 4. It consists of a glass tube of 8 mm inner diameter pressed via Kalrez-O-rings onto the template-stripped gold surface. The upper part is widened to increase the reaction volume. If needed, the chamber can accommodate a Pt100 thermosensor for temperature control and a platinum electrode for potentiostatic control. The glass tube is further equipped with a heating/cooling system and, on the top, with a Teflon seal for the injection of reagents. Inlet and outlet Teflon tubings provide, if needed, a well-defined gas phase, *e.g.*, of argon. With this

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[28] However, this procedure does not lead to increased flatness.

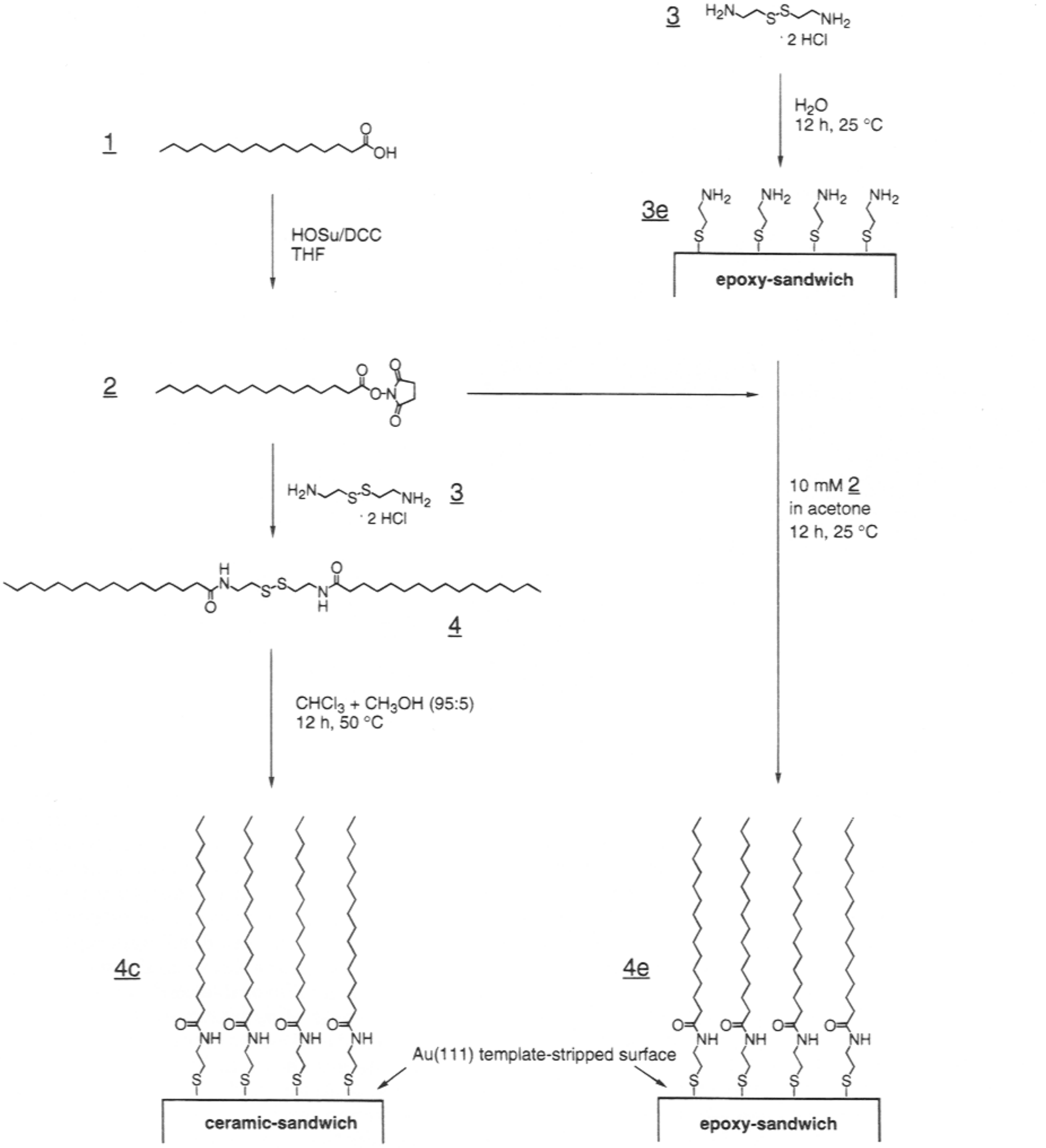

Figure 5. Reaction scheme of formation of SAMs **3e**, **4e**, and **4c**.

setup the preparation of self-assembled monolayers, the immobilization of biomolecules and their detection and modification *in situ*, and also special analyses can be carried out in the very same compartment under well-defined conditions.[29] Heating for thermal annealing of gold pits[30,31] or for domain fusion via decreasing chain tilt mismatches[32] within the monolayers during the self-assembly or after rinsing[33] is possible with temperatures as high as 100 °C, depending on the boiling points of the solvents. Multiple setups of 4, 10, and 20 units enable parallel experiments, which is essential for systematic investigations of SAMs, biochemical reactions, immunodetection, radiolabeling assays, etc., at these TSG interfaces. The reaction chambers can also be equipped with electrodes in order to perform electrodeposition of charged molecules onto the gold surface.

***In Situ* Synthesis of a SAM.**[34] As an example illustrating the usefulness of the combination template-stripped techniques with the reaction chamber, we synthesized a symmetric long-chain dialkyl disulfide **4**, which is insoluble in solvents other than tetrahydrofuran or chloroform–methanol (95:5, 50 °C) and the like. With these solvents the "ceramic-sandwich" is a must. In fact, with epoxy-glued TSGs, although the glass tube allows contact with the gold side only, over long periods solvents do diffuse through minute and occasional discontinuities in the gold film (mentioned above). This results in the disruption of the gold surface because of irreversible swelling of the epoxy glue.

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[29] The exposed Au area is 0.64 mm^2.

[30] McCarley, R. L.; Dunaway, D. J.; Willicut, R. J. *Langmuir* **1993**, *9*, 2775.

[31] Bucher, J. P.; Santesson, L.; Kern, K. *Langmuir* **1994**, *10*, 979.

[32] Delamarche, E; Michel, B.; Gerber, Ch.; Anselmetti, D.; Güntherodt, H.-J.; Wolf, H.; Ringsdorf, H. *Langmuir* **1994**, *10*, 2869.

[33] Delamarche, E.; Michel, B.; Kang, H.; Gerber, Ch. *Langmuir* **1994**, *10*, 4103.

[34] The goal of this publication is not to present a detailed characterization of SAM **4**. Therefore, this section is intentionally reduced. It is just a demonstration for *in situ* reactions (here extension) on a SAM chemisorbed on TSG.

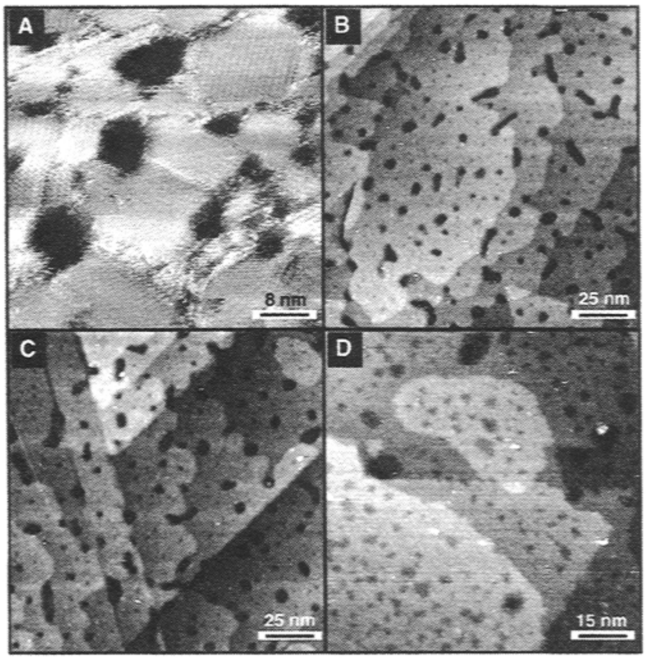

Figure 6. High resistance STM images of self-assembled monolayers chemisorbed on ultraflat TSGs. Tunnel parameters: $V_t = 1.2$ V; $I_t = 1-3$ pA. Key: (A) 1-dodecanethiol SAM prepared by immersion of a TSG platelet based on Epo-tek 377 in a 1 mM solution of the dodecanethiol in ethanol; (B) SAM **4c** directly prepared from compound **4** in the reaction chamber on a TSG platelet based on Cerastil C7; (C) SAM **4e** prepared *in situ* by acylation of SAM **3** in the reaction chamber supported TSG platelet based on Epo-tek 377; (D) SAM **3** directly prepared from compound **3** in the reaction chamber on a TSG platelet based on Epo-tek 377.

Figure 5 gives an overview of two routes to produce the same SAM **4** either directly on a "ceramic sandwich" **4c** (route of Figure 1D on Cerastil C7) or with an *in situ* synthesis on "epoxy-sandwich" **3e** (route of Figure 1A on Epo-tek 377) to SAM **4e**. That is, compound **4** was either synthesized *ex situ* by activation of palmitic acid (**1**) to the NHS ester **2**, followed by coupling to cystamine **3**, or *in situ* as SAM **4e** by first forming SAM **3e** and then extending it with **2**, both steps being carried out consecutively in the reaction chamber.

Figure 6A–D shows STM images of these SAMs taken at room temperature at ambient conditions in the constant-current mode. Hydrophobic alkanethiols with chain lengths of C_{10} to C_{18} are ideal model systems for studying the molecular structure of SAMs on a nm scale. In Figure 6A a high-resolution STM image of a 1-dodecanethiol SAM (49 × 49 nm^2) is shown, demonstrating the resolution of our STM measurements. Tunneling conditions with very high resistances ($\sim T\Omega$) were chosen to avoid tip penetration into the monolayer.[35,36] The obtained images confirm the nm scale structures of previous STM studies: [32,37-41] they exhibit domains which are molecularly resolved and separated by boundary lines, as well as missing rows and the well-known depressions, which are single-atom-deep etch pits and result from corrosion of gold by the thiols during the self-assembly process.[30,35,42-44] The commensurate overlayer structure consists of the hexagonal $(\sqrt{3} \times \sqrt{3})R30°$ lattice superimposed with $c(4 \times 2)$ superlattices with rectangular unit cells of $(3 \times 2\sqrt{3})d_{\mathrm{Au}}$ dimensions.

SAMs of alkanethiols are orderly and tightly packed, forming a solid-like all-trans assembly, which makes them accessible to a number of analytical methods (e.g., STM or diffraction techniques). Functional or bulky groups in the ω-position or within the alkyl chain as well as π-systems can disturb this regular packing. This results in a liquid-like assembly, which makes them difficult to resolve in detail by STM. The domain and molecular structures of the monolayers **3e**, **4e**, or **4c** could not be resolved by STM (Figure 6B–D). This is due to several reasons:

(i) the adsorption of cystamine **3** to form SAM **3e** (see ref 22) leads to a hydrophilic surface with amino end groups covered with coadsorbates and, most likely, forming hydrogen bonding induced surface reconstructions (see also ref 45). Furthermore, the length of **3** (approximately 4 Å) is not sufficient for adequate interchain stabilization; this leads to very loosely packed, liquid-like imperfect monolayers. But a monolayer was indeed formed, as shown by its ellipsometrical measured thickness of 3.8 Å; STM analyses of SAM **3e** (Figure 6D) reveal a complete coverage of the Au layer. The numerous depressions (2–8 nm in diameter) as mentioned above are equally distributed on the gold terraces. They are similar to those reported for SAMs of propanethiol on Au(111).[42] Their shape is also less smooth than those of 1-dodecanethiol (Figure 6A), which are often triangular smoothed.

(ii) SAM **4**, prepared either *in situ* as SAM **4e** by acylation of SAM **3e** with **2** or as SAM **4c** formed directly using compound **4** exhibits a break in the all-trans extended conformation due to the sp^2-carbon near the sulfur plane. However, it is rather likely that the C16 chains maintain van der Waals contacts by twisting the part between the sulfur and amide group into an energetically less favorable conformation. Ellipsometry gave a thickness of approximately 15 Å, which is 75% of the length of a C16-alkanethiol. The STM images of SAM **4c** and **4e** are shown in Figure 6B,C. We were not able to achieve atomic-scale resolution due to, we believe, imperfections and collapsed sites, as well as tip penetration (despite the high tunneling resistance) caused by the total thickness of the adsorbate. However, we want to point out that a comparison of Figure 6B,C shows no difference in topography and mean roughness of the monolayer and in number, size, and shape of the depressions. The depressions are similar in size as in Figure 6D and have a maximum depth of 10 Å. Hence, the *in situ* synthetic reaction had run to completion. SAM **3e** was completely formed beforehand (as in Figure 6D), since it was an efficient "initiation carpet" for SAM **4e**. Also, the type of glue underlying the TSGs had no influence on the structure of the monolayer.

(iii) All compounds described here are derivatives of dialkyl disulfides. The mode of adsorption of disulfides on Au(111) is still a matter of discussion. Recent results

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(35) Schönenberger, C.; Sondag-Huethorst, J. A. M.; Jorritsma, J.; Fokkink, L. G. J. *Langmuir* **1994**, *10*, 611.

(36) Salmeron, M.; Neubauer, G.; Folch, A.; Tomitori, M.; Ogletree, F.; Sautet, P. *Langmuir* **1993**, *9*, 3600.

(37) Widrig, C. A.; Alves, C. A.; Porter, M. D. *J. Am. Chem. Soc.* **1991**, *113*, 2805.

(38) Kim, Y.-T.; Bard, A. J. *Langmuir* **1994**, *8*, 1096.

(39) Anselmetti, D.; Baratoff, A.; Güntherodt, H.-J.; Delamarche, E.; Michel, B.; Gerber, Ch.; Kang, H.; Wolf, H.; Ringsdorf, H. *Europhys. Lett.* **1994**, *27*, 365.

(40) Poirier, G. E.; Tarlov, M. J. *Langmuir* **1994**, *10*, 2853.

(41) Bucher, J. P.; Santesson, L.; Kern, K. *Appl. Phys. A–Solids Surf.* **1994**, *59*, 135.

(42) Sondag-Huethorst, J. A. M.; Schönenberger, C.; Fokkink, L. G. J. *J. Phys. Chem.* **1994**, *98*, 6826.

(43) Häussling, L.; Michel, B.; Ringsdorf, H.; Rohrer, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 569.

(44) Han, T.; Beebe, T. P. *Langmuir* **1994**, *10*, 2705.

(45) For an STM study and MD simulations of a SH(CH$_2$)$_{12}$NH$_2$ monolayer see: Sprik, M.; Delamarche, E.; Michel, B.; Röthlisberger, U.; Klein, M. L., Wolf, H.; Ringsdorf, H. *Langmuir* **1994**, *10*, 4116.

from grazing incidence X-ray diffraction showed that the sulfur atoms of alkanethiols were bound as disulfides with a spacing of d_{ss} = 2.2 Å, indicating a nondissociated bonding state which would be inconsistent with a Au-thiolate bonding structure.[46] A gauche defect at the S−C bond allows the hexagonal hydrocarbon packing. The conformational changes caused by the disulfide chemisorption are additional factors which severely affect the two-dimensional arrangement of functionalized disulfides.

Conclusions

Template-stripped gold (TSG) thin films are highly suitable substrates for self-assembled monolayers whenever a very small mean roughness over large areas is needed. The techniques to prepare them are always based on gold deposition onto mica substrates with subsequent removal of the mica sheet to use the first, ultraflat gold atom layer. The procedures reported here differ in the ways of supporting the thin gold film. The different types of glues make them accessible to a variety of treatments and applications. In addition, we have shown that monolayers chemisorbed onto appropriate glued-supported TSGs can be successfully modified *in situ*. To perform this, we have developed multipurpose reaction chambers and have demonstrated the ease of an *in situ* synthesis with an acylation of a cysteamine SAM. This *in situ* synthesis does not detectably affect the topography of the gold surface.

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(46) Fenter, P.; Eberhardt, A.; Eisenberger, P. *Science* **1994**, *266*, 1216.