

Article

# Epoxy-Terminated Self-Assembled Monolayers Containing Internal Urea or Amide Groups

Michaël Ramin, Gwénaëlle Le Bourdon, Karine Heuze, Marie Degueil, Thierry Buffeteau, Bernard Bennetau, and Luc Vellutini

Langmuir, Just Accepted Manuscript • DOI: 10.1021/la5049375 • Publication Date (Web): 13 Feb 2015 Downloaded from http://pubs.acs.org on February 18, 2015

# Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.



Langmuir is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

# Epoxy-Terminated Self-Assembled Monolayers Containing Internal Urea or Amide Groups

Michaël A. Ramin, Gwénaëlle Le Bourdon, Karine Heuzé, Marie Degueil, Thierry Buffeteau, Bernard Bennetau, Luc Vellutini\*

Université Bordeaux, ISM, UMR 5255, F-33405 Talence, France

CNRS, ISM, UMR 5255, F-33405 Talence, France

AUTHOR EMAIL ADDRESS: luc.vellutini@u-bordeaux.fr

# **RECEIVED DATE:**

TITLE RUNNING HEAD: Epoxy-terminated SAMs

\* to whom correspondence should be addressed.

# ABSTRACT

We report the synthesis of new coupling agents with internal amide or urea groups possessing an epoxy-terminal group and trimethoxysilyl anchoring group. The structural characterizations of the corresponding self-assembled monolayers (SAMs) were performed by PM-IRRAS. The molecular assembly is mainly based on the intermolecular hydrogen-bonding between adjacent amide or urea groups in the monolayers. Due to the steric hindrance of amide or urea groups, the distance between the alkyl chains is too large to establish van der Waals interactions, inducing their disorder. The reactivity of the terminated-epoxy groups was successfully investigated through reaction with a fluorescent probe. We show that SAMs containing internal urea or amide groups exhibited a higher density of accessible epoxide groups than the corresponding long-chain ( $C_{22}$ ) glycidyl-terminated SAM.

KEYWORDS: Self-assembled monolayers; Silylated coupling agents; PM-IRRAS

#### Langmuir

Surface chemistry for immobilization of biomolecules onto solid surfaces plays a crucial role for many applications such as biosensors/biochips,<sup>1,2,3</sup> medical implants,<sup>4</sup> and drug/gene delivery carriers.<sup>5</sup> Biofunctional surfaces can be obtained if the immobilization process preserves the original recognition specificity of biomolecules and simultaneously allows strong binding with the surface.<sup>6</sup> Generally, the covalent way is favored because it leads to an irreversible bonding with the support, producing uniform surface coverage. In contrast, physical adsorption *via* intermolecular forces implies weak attachment of biomolecules, which may be removed by buffers when performing the assays.

Self-assembled monolayers (SAMs) based on the chemistry of the organosilanes are widely used to immobilize, in a covalent way, biomolecules such as DNA or proteins onto solid supports.<sup>7,8,9,10,11</sup> The chemical modification of Si/SiO<sub>2</sub> surfaces by epoxy groups represents an interesting approach for the covalent immobilization of biomolecules containing amino or thiol groups in one step.<sup>12</sup> Generally, the commercially available (3-glycidoxypropyl)trimethoxysilane (GPTS) is used to functionalize the silica surfaces for the chemical immobilization of small molecules,<sup>13,14,15</sup> polymers,<sup>16,17,18</sup> or biomolecules.<sup>19,20</sup> However, in the case of non atomically flat substrates, this short-chain arm can adopt various orientations and some of epoxide groups may not be accessible to the species to immobilize. Thus, in a recent study, we have shown that a very weak sensor response was measured when GPTS SAM was used to immobilize *E. coli* bacteria in liquid medium onto relatively rough silica surfaces (RMS  $\approx 5.5$  nm).<sup>21</sup> In contrast, a significant sensor response was measured when a very long-chain (C<sub>22</sub>) glycidyl-terminated SAM was used to immobilize bacteria on the surface. This improvement was attributed to a marked surface smoothing leading to a better efficiency of antigen-antibody recognition

 processes at the surface. The long-chain SAM affords a higher density of accessible epoxide groups to immobilize antibodies receptors.<sup>22</sup> However, it must be noticed that the synthetic routes leading to long-chain alkyl groups are time consuming and give relatively weak yields with poorly soluble silylated coupling agents. The urea group is widely recognized as an important building block in supramolecular architectures,<sup>23</sup> and the assemblies of organic/inorganic hybrid materials.<sup>24,25</sup> The intermolecular interactions between the organic fragments with urea groups provide hydrogen bonding and consequently offer an attractive route to self-direct the organization of molecular system.

Recently, we described a readily accessible way to synthesize functionalized ureido silylated coupling agents<sup>26</sup> by using isocyanate chemistry.<sup>27</sup> The presence of the urea group in the linear alkyl chain improves the solubility of coupling agents in organic solvents and urea moiety is easily generated by a condensation reaction of isocyanate with an amino group.<sup>28</sup> These functionalized ureido silylated coupling agents allow to prepare functionalized hydrogenbonding SAMs which can be grafted onto silica substrate. Indeed, the urea groups within the monolayer self-assemble strongly *via* hydrogen bonds. Conversely, the distance between the alkyl chains is too large to establish van der Waals interactions due to the steric hindering effect of urea groups, inducing the disorder of the alkyl chains.

Functionalized ureido silylated coupling agents have been used by our group for the formation of vinyl-terminated monolayer.<sup>29</sup> The successful chemical modifications to obtain vinyl-terminated SAMs allowed the covalent grafting of biomolecules without any degradation of the monolayer. In addition, we have shown that functionalized hydrogen-bonding SAMs lead to new platforms for the development of smooth silica-based surfaces, useful for structural biological studies.<sup>30</sup> Indeed, we have found that an acid-terminated ureido silylated compound

**ACS Paragon Plus Environment** 

#### Langmuir

based SAM was more suitable to maintain the structural integrity of viruses after its immobilization onto a soft organic SAM, rather than onto a mineral surface (mica, for example). The Atomic Force Microscopy (AFM) measurements, at the atomic level, show clearly that SAM surfaces allow imaging virus without distortion. Moreover, these SAM surfaces may prevent non-specific adsorption as previously reported with poly(ethyleneglycol) PEG-based monolayers.<sup>12,31,32</sup>

In this paper, we report the synthesis of two new coupling agents with internal amide and urea groups, possessing an epoxy-terminal group and a trimethoxysilyl anchoring group. Herein, we report the formation of epoxy-terminated monolayers possessing hydrogen-bonding network. The structural characterizations have been performed using PM-IRRAS.

# 2. EXPERIMENTAL SECTION

**2.1. Synthesis of Amido and Ureido Silylated Coupling Agents.** The synthesis of amido and ureido silylated coupling agents and their precursors are reported in Supporting Information (S2 to S8). All the compounds have been fully characterized by <sup>1</sup>H, <sup>13</sup>C and <sup>29</sup>Si NMR spectroscopy and by HRMS spectroscopy.

**2.2. Materials and substrates.** The SiO<sub>2</sub>/Au substrates were supplied by Optics Balzers AG. They correspond to Goldflex mirror with SiO<sub>2</sub> protection layer (GoldflexPRO, reference 200785). Their absolute reflectance was higher than 98% in the 1.2–12 µm spectral range. The thickness of the SiO<sub>2</sub> layer, measured by ellipsometry, was  $215 \pm 7$  Å, using a refractive index of 1.46 (I-elli2000 NFT ellipsometer,  $\lambda = 532$  nm). A homogeneous surface was observed by atomic force microscopy (AFM) with a rootmean-square (rms) roughness of 9 Å (Thermomicroscope Autoprobe CP Research, Park Scientific).

**2.3. Formation of Self-Assembled Monolayers (SAM-8 and SAM-15).** The substrates were cleaned and activated just before the grafting. They were treated successively with milli-Q water (18 M $\Omega$ .cm) and hot chloroform (10 min at least). Then, the substrates were exposed to UV-ozone (homemade apparatus,  $\lambda = 185-254$  nm) for 30 min and introduced into the silanization flask immediately. Trichloroacetic acid (TCA, 0.4 mg, 10 mol %) as catalyst was added to a solution of epoxy-terminated coupling agents (8 or 15) ( $2.5 \times 10^{-4} \text{ mol.L}^{-1}$ ) in anhydrous toluene (100 mL). This solution was introduced into the silanization flask at 18 °C under inert atmosphere. The substrates were immersed for 12 h. They were sonicated in toluene (5 min), chloroform (5 min) and milli-Q water (18.2 M $\Omega$ cm, 10 min) and then dried under vacuum for 10 min.

Contact angles were performed on a Krüss DSA 100 goniometer, at 20°C in static mode. The results correspond to the mean of least 3 measurements.

**2.4. Immobilization of dansylcadaverin onto SAM-8 and SAM-15.** The substrates functionalized by **SAM-8** and **SAM-15** were dipped into the solution of dansylcadaverin (10<sup>-4</sup> M) in toluene during 3 h at 37°C. The substrates were sonicated in toluene (5 min.) and milli-Q water (10 min.) and then dried under inert gas stream. The up-right widefield microscope was a Leica DMR (Leica Microsystems, Wetzlar, Germany) using objective HCX PL APO 63X oil (NA 1.32). The resolutive camera used on this system is a CoolSnap HQ (Photometrics, Tucson, USA). This system is equipped with an excitation (Ex/bp: 360/40) and emission (Em/bp: 528/38) filter wheels (Sutter instrument, Novato, USA). This system was controlled by MetaMorph software (Molecular Devices, Sunnyvale, USA).

#### Langmuir

2.5. PM-IRRAS experiments. PM-IRRAS spectra were recorded on a ThermoNicolet Nexus 670 FTIR spectrometer at a resolution of 4 cm<sup>-1</sup>, by coadding several blocks of 1500 scans (30 minutes acquisition time). All spectra were collected in a dry-air atmosphere after 30 min of incubation in the chamber. Experiments were performed at an incidence angle of 75° using an external homemade goniometer reflection attachment.<sup>33</sup> The infrared parallel beam was directed out of the spectrometer with an optional flipper mirror and made slightly convergent with a first BaF<sub>2</sub> lens. The IR beam passed through a BaF<sub>2</sub> wire grid polarizer (Specac) to select the p-polarized radiation and a ZnSe photoelastic modulator (PEM, Hinds Instruments, type III) which modulates the polarization of the beam at a high fixed frequency (74 KHz) between the parallel (p) and perpendicular (s) linear states. After reflection on the sample, the double modulated (in intensity and in polarization) infrared beam was focused with a second ZnSe lens onto a photovoltaic MCT detector (Kolmar Technologies, Model KV104) cooled at 77 K. In all experiments, the PEM was adjusted for a maximum efficiency at 2500 cm<sup>-1</sup> to cover the mid-IR range in only one spectrum. For calibration measurements, a second linear polarizer (oriented parallel or perpendicular to the first preceding the PEM) was inserted between the sample and the second ZnSe lens. This procedure was used to calibrate and convert the PM-IRRAS signal in

terms of the IRRAS signal (i.e.  $1 - \frac{R_p(d)}{R_p(0)}$ , where  $R_p(d)$  and  $R_p(0)$  stand for the p-polarized

reflectance of the film/substrate and bare substrate systems, respectively).<sup>34</sup>

**2.6.** ATR experiments, Determination of the optical constants of 8 and 15, Spectral simulation of the IRRAS spectra of SAM-8 and SAM-15 for a compact monolayer. The ATR spectra of compound 8 and 15 were recorded with a ThermoNicolet Nexus 670 FTIR spectrometer equipped with a liquid nitrogen cooled narrow-band mercury cadmium telluride

(MCT) detector using a Silver-Gate (germanium crystal) ATR accessory (Specac). The electric field of the infrared beam was polarized either perpendicular (s-polarized) or parallel (p-polarized) to the plane of incidence with a  $BaF_2$  wire grid polarizer (Specac). Each spectrum was obtained, at a resolution of 4 cm<sup>-1</sup>, by coadding 500 scans.

The optical constants (refractive index  $n(\overline{v})$  and extinction coefficient  $k(\overline{v})$ ) of compounds 8 and 15 have been determined from polarized ATR spectra, using the procedure decribed by Dignam et al.<sup>35</sup> The computer program used to calculate the IRRAS spectra for a compact monolayer of SAM-8 and SAM-15 deposited onto SiO<sub>2</sub>/Au substrates is based on the Abeles' matrix formalism,<sup>36, 37</sup> which has been generalized for anisotropic layers.<sup>38</sup> Several parameters must be fixed in the program such as the the thickness of the SAMs (set to 33 and 35 Å for SAM-8 and SAM-15, respectively), the angle of incidence (set to 75°) and the polarization of the infrared beam. The p-polarized reflectance of the covered  $R_p(d)$  and bare  $R_p(0)$ substrates have been calculated using the spectral dependence of the optical constants of compounds 8 and 15 of gold.<sup>39</sup>

# **3. RESULTS AND DISCUSSION**

# 3.1. Synthesis of Amido and Ureido Silylated Coupling Agents.

The synthesis of a novel trimethoxysilane **8** with an amide group in the alkyl chain is reported in Scheme 1. It is performed in 8 steps from commercially available starting compounds.

#### Langmuir



Scheme 1. Synthesis of amido silylated coupling agent (8). a) Pyridine, Ac<sub>2</sub>O, 60 °C, 3 h. b) OsO<sub>4</sub> (0.01 equiv), Oxone<sup>®</sup> (4 equiv), DMF, 3 h, TA. c) NaN<sub>3</sub> (1.2 equiv), DMF. d) PPh<sub>3</sub> (1.5 equiv), H<sub>2</sub>O, THF, 40°C, 3 h. e) DCC (1.1 equiv), NHS (1.1 equiv), 4 (1.4 equiv), CH<sub>2</sub>Cl<sub>2</sub>. f) K<sub>2</sub>CO<sub>3</sub>, MeOH, H<sub>2</sub>O. g) NaH (1.3 equiv), epichlorhydrine (5.5 equiv), THF. h) HSi(OMe)<sub>3</sub> (5 equiv), cat. Karstedt (0.025 equiv Pt), 65 °C, 20 h.

The unsaturated alcohol has been acetylated by treatment with acetic anhydride in pyridine to avoid the intramolecular lactonization and the esterification of compound **1** in the following steps. The oxidative cleavage of the terminal alkene to carboxylic acid (compound **2**) has been performed one pot, in high yield, with Osmium(VIII) oxide (called also tetroxide osmium) in catalytic amount using Oxone<sup>®</sup> (KHSO<sub>5</sub>.  $\frac{1}{2}$  KHSO<sub>4</sub>.  $\frac{1}{2}$  K<sub>2</sub>SO<sub>4</sub>) as co-oxidant.<sup>40</sup> Nucleophilic displacement of the bromide from the bromo-alkene with azide afforded intermediate **3**, which was easily converted to the primary amine **4** using the Staudinger reduction.<sup>41</sup> The coupling reaction between the acid **2** and amine **4** derivatives to generate the amido compound **5** was performed by using the carbodilimide chemistry to activate the carboxylic acid group into an active ester.<sup>42</sup> Then, the acetyl group of compound **5** was deprotected using K<sub>2</sub>CO<sub>3</sub> in methanol to obtain the compound **6** in high yield (70%). The

**ACS Paragon Plus Environment** 

intermediate alcohol **6** was reacted with epichlorhydrine and NaH under anhydrous conditions in one step procedure, yielding 7 (28%).<sup>22</sup> To obtain the amido silylated coupling agent **8**, the addition of the Si(OMe)<sub>3</sub> group was achieved by hydrosilylation reaction of the unsaturated precursor 7 with a large excess of HSi(OMe)<sub>3</sub> in the presence of Karstedt's catalyst.<sup>29</sup>

The synthesis of novel epoxy alkoxyorganosilane with an urea group in the alkyl chain required 7 steps (Scheme 2).



Scheme 2. Synthesis of ureido silylated coupling agent (15). a) TBDPSCl (1.5 equiv), imidazole (1.2 equiv), CH<sub>2</sub>Cl<sub>2</sub>. b) NaN<sub>3</sub> (1.2 equiv), DMF. c) H<sub>2</sub>, Pd/C, AcOEt. d) 10-isocyanatodecene (0.9 equiv), CH<sub>2</sub>Cl<sub>2</sub>. e) TBAF (1.8 equiv), THF. f) NaH (1.3 equiv), epichlorhydrine (5.5 equiv), THF. g) HSi(OMe)<sub>3</sub> (5 equiv), cat. Karstedt (0.025 equiv en Pt), 65 °C, 20 h.

The hydroxyl group of the 11-bromo-1-undecanol was protected as tertbutyldiphenylsilyl (TBDPS) ether **9** to avoid undesired further reactions. The azide derivative **10** was obtained from an azide substitution of halide compound **9**. The azide compound **10** constitutes an useful precursor of amine since it can be easily reduced in the corresponding amino compound **11** in high yield (84%) by a catalytic hydrogenation over 10 % Pd/C in AcOEt. Page 11 of 24

#### Langmuir

It is interesting to note that the silyl protective group TBDPS was stable during hydrogenation reaction if a non-protic solvent was used.<sup>43</sup> Then, this amine compound **11** has been coupled with 10-isocyanatodecene to form the urea derivative **12** in high yield (80 %). The cleavage of the TBDPS protective group of **12** was performed easily to provide alcohol **13** by treatment with tetra-butylammonium fluoride (TBAF) in THF.<sup>44</sup> The intermediate alcohol **13** was reacted with epichlorhydrine and NaH under anhydrous conditions, yielding **14** (21%). Finally, the ureido silylated coupling agent **15** was obtained by hydrosilylation reaction of the unsaturated precursor **14** with a large excess of HSi(OMe)<sub>3</sub> in the presence of Karstedt's catalyst.

FTIR spectra of compounds 7, 8, 14, 15 are reported in Figures 1A and 1B. These spectra are presented in the region of the amide I ( $v_{C=O}$ ) and amide II ( $\delta_{NH}+v_{C-N}$ ) modes, at around 1650  $cm^{-1}$  and 1550  $cm^{-1}$ , respectively. The presence of hydrogen-bonding between the molecules is revealed by the wavenumber difference ( $\Delta v$ ) between the amide 1 and 2 modes. Indeed, the smaller is the wavenumber difference, the stronger are the hydrogen bonds between adjacent amide or urea groups. A  $\Delta v$  value of 176 cm<sup>-1</sup> has been measured for a diluted solution of 7 in CCl<sub>4</sub>, showing that any intermolecular hydrogen-bonding is possible. This value decreases up to 89 and 99 cm<sup>-1</sup> in the solid state for the alkene precursor 7 and the silvlated amido coupling agent  $\mathbf{8}$ , respectively. The higher value measured for compound  $\mathbf{8}$  is certainly due to the presence of the -Si(OMe)<sub>3</sub> group which induces steric hindrance and consequently increases the distance between amide groups. For the ureido derivative, a  $\Delta v$  value of 131 cm<sup>-1</sup> has been observed for a diluted solution of 14 in CCl<sub>4</sub>, associated to weak intermolecular hydrogen-bonding. This  $\Delta v$ value decreases up to 37 and 44 cm<sup>-1</sup> in the solid state for the alkene precursor 14 and the silvlated ureido coupling agent 15, respectively. As with compound 8, the addition of bulky silvl group to obtain the ureido coupling agent 15 induces weaker hydrogen bonds. The ability to self-

assemble through intermolecular hydrogen-bonding seems to be better for the silvlated ureido coupling agent **15** (44 cm<sup>-1</sup>) than for the amide analog compound **8** (99 cm<sup>-1</sup>).



Figure 1. A) IR spectra in the 1800-1450 cm<sup>-1</sup> spectral range of 7 in CCl<sub>4</sub> solution at low concentration ( $5.10^{-3}$  M) and in the solid state for 7 and 8 compounds. B) IR spectra in the 1800-1500 cm<sup>-1</sup> spectral range of 14 in CCl<sub>4</sub> solution at low concentration ( $5.10^{-3}$  M) and in the solid state for 14 and 15 compounds.

**3.2.** Characterization of Epoxy-Terminated Monolayers. Epoxy-terminated amido (SAM-8) and ureido (SAM-15) monolayers (Scheme 3) were characterized by wettability measurements. Contact angle values of  $72^{\circ}\pm1^{\circ}$  and  $70^{\circ}\pm2^{\circ}$  (with water) were measured for SAM-8 and SAM-15. These values are similar to those found in the literature for an organosilicon coupling agent containing a long hydrocarbon chain (C<sub>22</sub>) and a glycidyl end-group ( $72^{\circ}\pm1^{\circ}$ ).<sup>22</sup> They are much higher than that reported in the literature ( $55^{\circ}\pm10^{\circ}$ ) for a GPTS monolayer.<sup>45</sup> These results show that the glycidyl-terminated long-chain SAM possesses a

#### Langmuir

relatively high hydrophobic character, whereas GPTS monolayers exhibit a poor barrier effect to water.



Scheme 3. Schematic representation of the epoxy-terminated amido (SAM-8) and epoxy-terminated ureido (SAM-15) self-assembled monolayers.

The PM-IRRAS spectra of epoxy-terminated monolayers are presented in Figure 2 in the 3200-2600 cm<sup>-1</sup> region, characteristic to the methylene stretching vibrations, and in the 1700-1400 cm<sup>-1</sup> region, characteristic to the amide I and II modes. The methylene stretching region provides specific information about the conformation and van der Waals interactions of the alkyl chains. No significant difference appears in the wavenumbers of the antisymmetric ( $v_aCH_2$ ) and symmetric ( $v_sCH_2$ ) stretching vibrations of the methylene groups observed respectively at 2928 and 2855 cm<sup>-1</sup>, revealing a disorder of the alkyl chains in the monolayers for the two epoxy-terminated monolayers.<sup>46</sup> In previous papers concerning functionalized hydrogen-bonding SAMs, we have shown that the distance between the alkyl chains is too large (4.5–5 Å) to

establish van der Waals interactions due to the presence of urea groups.<sup>26,29</sup> This study shows that the presence of amide groups is also not favorable to form well ordered SAMs.



**Figure 2.** PM-IRRAS spectra of the silvlated amido (A) and ureido (B) coupling agents grafted onto SiO<sub>2</sub>/Au substrate.

The two broad bands observed at 1649 (1638) cm<sup>-1</sup> and at 1550 (1571) cm<sup>-1</sup>, attributed to the amide I ( $v_{C=O}$ ) and amide II ( $\delta_{NH}+v_{C-N}$ ) modes of amide (urea) group, respectively, confirm that the molecules are grafted onto the surface. The width of these bands is relatively broad, indicating a distribution of the hydrogen bonds in the SAMs. The  $\Delta v$  values of 99 cm<sup>-1</sup> and 67 cm<sup>-1</sup> are indicative of strong association of the amide and urea groups by hydrogen bonds in the epoxy-terminated monolayers, respectively. These values appeared significantly lower than those measured for the free amide (176 cm<sup>-1</sup>) and urea (131 cm<sup>-1</sup>) groups in diluted CCl<sub>4</sub> solution, respectively. From this result and considering the disorder of the alkyl chains, the major driving forces operating in the molecular assembly are mainly based on the strong intermolecular hydrogen-bonding between the amide or urea groups in the monolayers. It is noteworthy that the monolayers containing internal urea or amide groups are formed only after 12 hours of immersion in the silanization solution whereas the grafting takes at least 20 hours for monolayers

#### Langmuir

possessing long alkyl chains.<sup>22</sup> This feature is certainly due to the fact that using hidden polar functionalities increased the solubility in organic solvent and also the affinity with hydrophilic surfaces.

To estimate the surface coverage of **SAM-8** and **SAM-15** onto SiO<sub>2</sub>/Au surface, the IRRAS spectra expected for a compact monolayer of the molecules **8** and **15** were calculated (Supporting information, S9 to S12). This calculation has been performed using the isotropic optical constants of **8** and **15** determined from polarized ATR spectra (Supporting information, S9) and considering a thickness of 33 and 35 Å for **SAM-8** and **SAM-15**, respectively.<sup>47</sup> The simulated IRRAS spectra of **SAM-8** and **SAM-15** are reported in Supporting information (S12) to be compared with the experimental ones. The intensities of the experimental and calculated spectra were in the same order of magnitude, revealing a complete surface coverage of **SAM-8** and **SAM-15** onto SiO<sub>2</sub>/Au surface. The relative intensity of the amide 1 and amide 2 bands calculated for the ureido silylated coupling agent didn't reproduce that observed in the experimental IRRAS spectrum due to the preferential orientation of the C=O groups parallel to the surface to form intermolecular hydrogen-bonding between adjacent urea groups. In contrast, for amido silylated coupling agent, the C=O groups seem to be randomly oriented.

Evidence of the presence of reactive epoxide groups on the surface was provided in epifluorescence microscopy using a fluorescent probe. The dansylcadaverin ( $\lambda_{ex} = 335$  nm,  $\lambda_{em} =$ 508 nm in methanol) was grafted onto the surface via a nucleophilic attack of the epoxide groups by the amino group and ring-opening condensation.<sup>22</sup> For both surfaces, the fluorescence intensity is homogenously distributed on the whole surfaces, showing that the fluorophore was homogeneously distributed on the surfaces. As expected, blank experiment, consisting of the direct treatment of the silica surface by the fluorescent probe, only led to a very weak

fluorescence signal (lower than 100 RFU) that could be assimilated to the background. As shown in Figure 3, the signal intensity was slightly higher for **SAM-15** (close to 800 RFU) than for **SAM-8** (680 RFU), indicating that urea-SAM exhibited a higher density of accessible surface epoxide groups. This result is consistent with the higher intensity of the alkyl and amide bands observed in the PM-IRRAS spectra for **SAM-15** than for **SAM-8**. Interestingly, the epoxyterminated long chain ( $C_{22}$ ) SAM exhibits a low reactivity (600 RFU) which may be due to the high degree of ordering of molecules inducing a steric hindrance between the epoxy groups.<sup>22</sup> The presence of the amide or urea groups in the middle of the alkyl chains does not affect the reactivity of terminal-epoxy groups. The better reactivity of **SAM-15** could be related to the stronger intermolecular hydrogen-bonding allowing a better cohesion of molecules and limiting locally the conformational constraints.



**Figure 3.** Diagram of mean fluorescence intensities for epoxy-terminated monolayers grafted with dansylcadaverin as a fluorescent probe (each point corresponds to the mean value of four replicates and the errors bars represent  $\pm$  SD).

#### Langmuir

# 4. CONCLUSION

In this paper, we described the successful synthesis of silylated coupling agents with internal amide or urea moieties possessing epoxy-terminal groups. These amido and ureido silylated coupling agents have shown their ability to autoassociate through hydrogen bonds. The PM-IRRAS characterization confirms the grafting of these two silylated coupling agents to afford the corresponding epoxy-terminated monolayers. The molecular assembly is mainly based on the intermolecular hydrogen-bonding between the adjacent amide or urea groups in the monolayers which favors the kinetic of the formation of the SAMs. The reactivity of the terminated-epoxy groups was successfully investigated through reaction with a fluorescent probe, giving evidence that SAMs containing internal urea or amide groups exhibited a higher density of accessible epoxide groups than for the corresponding long-chain ( $C_{22}$ ) glycidyl-terminated SAM. These functionalized hydrogen-bonding SAMs show promise as a novel platform for the biofunctionalization of surfaces in one step.

# AUTHOR INFORMATION

# **Corresponding Author**

\* E-mail: <u>luc.vellutini@u-bordeaux.fr</u>

# Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENT

The authors gratefully acknowledge the financial supports of the "Ministère de la Recherche" and the CNRS. The microscopy was done in the Bordeaux Imaging Center a service unit of the

CNRS-INSERM and Bordeaux University, member of the national infrastructure France BioImaging. The help of Christel Poujol is acknowledged.

#### SUPPORTING INFORMATION AVAILABLE

Synthesis of amido and ureido silylated coupling agents and their precursors. P- and s-polarized ATR (germanium crystal) spectra of compounds 8 and 15. Anisotropic optical constants of compounds 8 and 15. Isotropic optical constants of compounds 8 and 15. Comparison of experimental IRRAS spectra of SAM-8 and SAM-15 with those calculated for compact monolayers of 8 and 15, respectively. This material is available free of charge via the Internet at <a href="http://pubs.acs.org">http://pubs.acs.org</a>.

#### REFERENCES

(1) Sassolas, A.; Leca-Bouvier, B. D.; Blum, L. J. DNA Biosensors and Microarrays. *Chem. Rev.* **2008**, *108*, 109-139.

(2) Rusmini, F.; Zhong, Z.; Feijen, J. Protein Immobilization Strategies for Protein Biochips. *Biomacromolecules* **2007**, *8*, 1775-1789.

(3) Tjong, V.; Tang, L.; Zauscher, S.; Chilkoti, A. "Smart" DNA interfaces. *Chem. Soc. Rev.*, **2014**, *43*, 1612-1626.

(4) Früh, V.; IJerman, P.; Siegal, G. How to Catch a Membrane Protein in Action: A Review of Functional Membrane Protein Immobilization Strategies and Their Applications. *Chem. Rev.* 2011, *111*, 640-656.

(5) Langer, R. Perspectives: Drug delivery- drugs on target. Science 2001, 293, 58-59.

(6) Wong, L. S.; Khan, F.; Micklefield, J. Selective Covalent Protein Immobilization: Strategies and Applications. *Chem. Rev.* **2009**, *109*, 4025-4053.

#### Langmuir

(7) Jonkheijm, P.; Weinrich, D.; Schröder, H.; Niemeyer, C. M.; Waldmann, H. Chemical Strategies for Generating Protein Biochips. *Angew. Chem. Int. Ed.* **2008**, *47*, 9618-9647.

(8) Pirrung, M. How to Make a DNA Chip. Angew. Chem. Int. Ed. 2002, 41, 1276-1289.

(9) Schaeferling, M.; Schiller, S.; Paul, H.; Kruschina, M.; Pavlickova, P.; Meerkamp, M.; Giammasi, C.; Kambhampati, D. Application of self-assembly techniques in the design of biocompatible protein microarray surfaces. *Electrophoresis* **2002**, *23*, 3097-3105.

(10) Debasis Samanta; Amitabha Sarkar Immobilization of bio-macromolecules on self-assembled monolayers: methods and sensor applications. *Chem. Soc. Rev.* **2011**, *40*, 2567-2592.

(11) Vashist, S. K.; Lam, E.; Hrapovic, S.; Male, K. B.; Luong, J. H. T. Immobilization of Antibodies and Enzymes on 3-Aminopropyltriethoxysilane-Functionalized Bioanalytical Platforms for Biosensors and Diagnostics. *Chem. Rev.* **2014**, *114*, 11083-11130.

(12) Böcking, T.; Kilian, K. A.; Gaus, K.; Gooding, J. Single-Step DNA Immobilization on Antifouling Self-Assembled-Monolayers Covalently Bound to Silicon (111). *Langmuir* 2006, *22*, 3494-3496.

(13) Ding, L.; Fang, Y.; Blanchard, G. J. Probing the Effects of Cholesterol on Pyrene-Functionalized Interfacial Adlayers. *Langmuir* **2007**, *23*, 11042-11050.

(14) Ding, L.; Fang, Y. Chemically assembled monolayers of fluorophores as chemical sensing materials. *Chem. Soc. Rev.* **2010**, *39*, 4258 - 4273.

(15) Gökcen Birlik Demirel, Nursel Dilsiz, Mehmet, Cakmak and Tuncer Caykara Molecular design of photoswitchable surfaces with controllable wettability. *J. Mater. Chem.* **2011**, *21*, 3189-3196.

(16) Elender, G.; Kühner, M.; Sackmann, E. Functionalisation of Si/SiO<sub>2</sub> and glass surfaces with ultrathin dextran films and deposition of lipid bilayers. *Biosens. Bioelec.* **1996**, *11*, 565-577.

(17) Lusinov, I.; Julthongpiput, D.; Liebmann-Vinson, A.; Cregger, T.; Foster, M. D.; Tsukruk,
V. V. Epoxy-Terminated Self-Assembled Monolayers: Molecular Glues for Polymer Layers. *Langmuir* 2000, *16*, 504-516.

(18) Demirel, G. B.; Coşkun, S.; Kalkan, M.; Caykara, T. Preparation of a Novel Polymer-Modified Si Surface for DNA Immobilization. *Macromol. Biosci.* **2009**, *9*, 472-479.

(19) Cloarec, J. P.; Deligianis, N.; Martin, J. R.; Lawrence, I.; Souteyrand, E.; Polychronakos,
C.; Lawrence, M. F. Immobilization of homooligonucleotide probe layers onto Si/SiO<sub>2</sub> substrates: characterization by electrochemical impedance measurements and radiolabelling. *Biosens. Bioelec.* 2002, *17*, 405-412.

(20) Massé, P.; Vellutini, L.; Bennetau, B.; Ramin, M. A.; Fournel F.; Blanc, L.; Dejous, C.; Rebière, D.; Weisbecker, P.; Pillot J.-P. Chimie douce route to novel acoustic waveguides based on biphenylene-bridged silsesquioxanes. *J. Mater. Chem.* **2011**, *21*, 14581-14586.

(21) Dinh, D. H.; Pascal, E.; Vellutini, L.; Bennetau, B.; Rebière, D.; Dejous, C.; Moynet, D.; Belin, C.; Pillot J.-P. Novel optimized biofunctional surfaces for Love mode surface acoustic wave based immunosensors. *Sensors and Actuators B* **2010**, *146*, 289-296.

#### Langmuir

(22) Dinh, D. H.; Vellutini, L.; Bennetau, B.; Dejous, C.; Rebière, R.; Pascal, E.; Moynet, D.; Belin, C.; Desbat, B.; Labrugère, C.; Pillot, J-P. Route to Smooth Silica-Based Surfaces Decorated with Novel Self-Assembled Monolayers (SAMs) Containing Glycidyl-Terminated Very Long Hydrocarbon Chains. *Langmuir* **2009**, *25*, 5526-5535.

(23) MacDonald, J. C.; Whitesides, G. M. Solid-state Structures of Hydrogen-Bonded Tapes Based on Cyclic Secondary Diamides. *Chem. Rev.* **1994**, *94*, 2383-2420.

(24) Pichon, B. P.; Wong Chi Man, M.; Dieudonné, P.; Bantignies, J.-L.; Bied, C.; Sauvajol, J.-L.; Moreau J. J. E. Size and Shape Dependence of Organo-Interconnected Silsesquioxanes through Hydrolysis-Condensation Reaction Conditions: Nanotubes, Spheres and Films. *Adv. Funct. Mater.* **2007**, *17*, 2349-2355.

(25) Moreau, J.J.E., Vellutini, L., Bied, C., Man, M.W.C. New approach for the organisation and the shaping of organo-bridged silicas: An overview. *J. Sol-Gel Sci. Technol.* **2004**, *31*, 151-156.

(26) Ramin, M. A.; Le Bourdon, G.; Daugey, N.; Bennetau, B.; Vellutini, L.; Buffeteau, T. PM-IRRAS Investigation of Self-Assembled Monolayers Grafted onto SiO<sub>2</sub>/Au Substrates. *Langmuir* 2011, *27*, 6076-6084.

(27) Delebecq, E.; Pascault, J.-P.; Boutevin, B.; Ganachaud, F. On the Versatility of Urethane/Urea Bonds: Reversibility, Blocked Isocyanate, and Non-isocyanate Polyurethane. *Chem. Rev.* 2013, *113*, 80-118.

(28) Nam, H.; Granier, M.; Boury, B.; Park, S. Y. Functional Organotrimethoxysilane Derivative with Strong Intermolecular  $\pi$ – $\pi$  Interaction: One-Pot Grafting Reaction on Oxidized Silicon Substrates. *Langmuir* **2006**, *22*, 7132-7134.

(29) Ramin, M. A.; Le Bourdon, G.; Heuzé, K.; Degueil, M.; Belin, C.; Buffeteau, T.; Bennetau,
B.; Vellutini, L. Functionalized Hydrogen-Bonding Self-Assembled Monolayers Grafted onto
SiO<sub>2</sub> Substrates. *Langmuir* 2012, *28*, 17672-17680.

(30) Meillan, M.; Ramin, M. A.; Buffeteau, T.; Marsaudon, S.; Odorico, M.; Chen, S. W.; Pellequer, J.-L.; Degueil, M.; Heuzé, K.; Vellutini, L.; Bennetau, B. Self-assembled monolayer for AFM measurements of Tobacco Mosaic Virus (TMV) at the atomic level. *RSC advances* **2014**, *4*, 11927-11930.

(31) Schenk, F. C.; Boehm, H.; Spatz, J. P.; Wegner, S. V. Dual-Functionalized Nanostructured Biointerfaces by Click Chemistry. *Langmuir* **2014**, *30*, 6897-6905.

(32) Banerjee, I.; Pangule, R. C.; Kane, R. S. Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. *Adv. Mater.* **2011**, *23*, 690-718.

(33) Buffeteau, T.; Desbat, B.; Turlet, J.-M. Polarization ModulationFT-IR Spectroscopy of Surfaces and Ultra-thin Films: Experimental Procedure and Quantitative Analysis. *Appl. Spectrosc.* **1991**, *45*, 380-389.

(34) Buffeteau, T.; Desbat, B.; Blaudez, D.; Turlet, J.-M. Calibration Procedure to Derive IRRAS Spectra from PM-IRRAS Spectra. *Appl. Spectrosc.* **2000**, *54*, 1646-1650.

(35) Dignam, M. J.; Mamicheafara, S. Determination of the Spectra of the Optical Constants of Bulk Phases via Fourier Transform ATR. *Spectrochim. Acta, Part A* **1988**, *44*, 1435-1442.

2
3
4
5
6
7
1
8
9
10
10
11
12
13
11
14
15
16
17
18
10
19
20
21
22
~~
23
24
25
26
20
27
28
29
20
30
31
32
33
24
34
35
36
37
20
38
39
40
<u>4</u> 1
40
42
43
44
45
40
40
47
48
49
50
51
52
53
50
54
55
56
57
50
20
59
20

(36) Hansen, W. N. Electric Fields Produced by the Propagation of Plane Coherent Electromagnetic Radiation in a Stratified Medium. *J. Opt. Soc. Am.* **1968**, *58*, 380-390.

(37) Buffeteau, T.; Desbat, B. Thin-Film optical Constants Determined from Infrared Reflectance and Transmittance Measurements. *Appl. Spectrosc.* **1989**, *43*, 1027-1032.

(38) Yamamoto, K.; Ishida, H. Interpretation of reflection and Transmission Spectra for Thin Films. *Appl. Spectrosc.* **1994**, *48*, 775-787.

(39) Palik, E. D. Handbook of Optical Constants of Solids; Academic Press: New York, 1985.

(40) Travis, B. R.; Narayan, R. S.; Borhan, B. Osmium Tetroxide-Promoted Catalytic Oxidative Cleavage of Olefins: An Organometallic Ozonolysis. *J. Am. Chem. Soc.* **2002**, *124*, 3824-3825.

(41) Koziara, A.; Osowska-Pactwicka, K.; Zawadzki, S.; Zwierzak, A. One-Pot Transformation of Alkyl Bromides into Primary Amines via the Staudinger Reaction. *Synthesis* **1985**, *2*, 202-204.

(42) Montalbetti, C. A.; Falque, V. Amide bond formation and peptide coupling. *Tetrahedron* 2005, *61*, 10827-10852.

(43) Sajiki, H.; Ikawa, T.; Hattori, K.; Hirota, K. A remarkable solvent effect toward the Pd/C-catalyzed cleavage of silyl ethers. *Chem. Commun.* **2003**, *5*, 654-655.

(44) Corey, E. J.; Venkateswarlu, A. Protection of hydroxyl groups as tert-butyldimethylsilyl derivatives. *J. Amer. Chem. Soc.* **1972**, *94*, 6190-6191.

(45) Tsukruk, V. V.; Luzinov, I.; Julthongpiput, D. Sticky Molecular Surfaces: Epoxysilane Self-Assembled Monolayers. *Langmuir* **1999**, *15*, 3029-3032.

(46) Snyder, R, G.; Strauss, H, L.; Elliger, C. A. Carbon-hydrogen stretching modes and the structure of n-alkyl chains. 1. Long, disordered chains. *J. Phys. Chem.* **1982**, *86*, 5145-5150.

(47) These thicknesses correspond to the full length of 8 and 15, considering an all-trans conformation of the alkyl chains.

SYNOPSIS

