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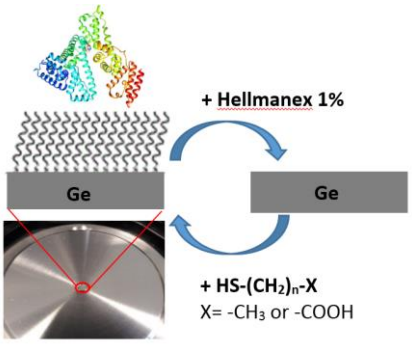
ABSTRACT: The functionalization of germanium by SAMs is commonly used to passivate the surface and thus prevent its oxidation. This functionalization can also be very useful to study specific interactions for instance in biosensors using protein or enzyme recognition. Several functionalization methods exist but no method has up to now been described to regenerate the Ge surface. A procedure for regenerating the surface of Ge after functionalization by SAMs is proposed in this article. The characterization of the functionalized and regenerated Ge surface by contact angle, XPS and ATR-FTIR, allows to establish that the protocol is robust. Hydrophilic SAMs (HS-$(CH_2)_n$-COOH, HS-$(CH_2)_n$-OH) and hydrophobic SAMs (HS-(CH_2)n-CH_3) of different chain lengths (n=5 or 10) were tested. After ten cycles of functionalization and regeneration, the surface of germanium has the same wettability, with a small decrease in its oxidation. As an example of interest, this method allows to study the structure of protein adsorbed on biomimetic surfaces (type SAMs) by ATR-FTIR with a reusable commercial Ge crystal following the elimination of SAMs with a process retaining the native properties of the Ge.



■ INTRODUCTION

Self-assembled monolayers (SAMs) of organic molecules are commonly used to modify chemical and physical properties of surfaces. The modified surfaces are then used in various applications such as metal corrosion barrier, biosensors and biomaterials. SAMs are mainly grafted on inorganic materials (glass, Au, Si, ZnSe, Ge). Grafting of Ge and ZnSe surfaces can be followed by ATR-FTIR spectroscopy, as well as enzyme or protein immobilization. Several protocols are described for grafting Ge. The first and crucial step is to remove germanium oxides from the surface before functionalization. Hydrofluoric acid (HF)[1], aqueous halogenic acids (HCl, HBr, or HI) or hydrogen peroxide (H_2O_2)[2-5] were used to achieve this step. After removing oxides, the grafting was obtained by simple immersion of Ge surface in thiol molecules solution. Then stable SAMs of organic molecules are formed on Ge surface with fairly good stability.[6] Hohman *et al.* developed a simple and robust method of molecular self-assembly on germanium in a single step.[7] SAMs are deposited directly on germanium surface from a solution of alkane thiol in a 1:1 mixture of water and ethanol. No pretreatment of Ge surface was required. They demonstrated that, while the presence of water allows to remove oxides from Ge surface , the presence of the ethanol leads to solubilize the alkane thiol and to form the monolayer on the Ge surface.[7] All the data reported in the literature described various processes of grafting, but no data are reported for Ge crystal regeneration. This is an important point in order to be able to perform surface functionalization on commercial ATR-FTIR set-ups.

In this paper, we describe a simple protocol to functionalize Ge crystal by thiols, based on the Hohman process and to regenerate it to be re-used. The grafting and desorption of the self-assembled monolayer of thiols were followed by X-ray photoelectron spectroscopy (XPS) and by ATR-FTIR spectroscopy. As example, the modifications of secondary structure of Human Serum Albumin (HSA) adsorbed on SAMs with various hydrophilic/hydrophobic properties were studied by ATR-FTIR spectroscopy. The developed protocol paves the way of an easy method to probe molecular interactions or structural modifications of molecules adsorbed on modified surface in liquid media.

■ EXPERIMENTAL SECTION

Materials. Ge (100) plates were purchased from Kirchheim Optique. Human Serum Albumin (HSA), Hellmanex and thiol compounds ($HS(CH_2)_5COOH$, $HS(CH_2)_{10}COOH$, $HS(CH_2)_{10}OH$, $HS(CH_2)_5CH_3$, $HS(CH_2)_{10}CH_3$) were purchased from Sigma.

Functionalization and regeneration of Ge surface.

Before functionalization, germanium plate was deposited in Hellmanex 1% 10 min and rinsed with milliQ water. The plate was then incubated in thiol solution at 5mM (water : ethanol 50 :50) for one night. Finally, the surface was cleaned with ethanol and dried under nitrogen flux.

Removing of thiol monolayer from germanium surface. The Ge surface was regenerated by a 1% Hellmanex solution for 1 hour, then rinsed with milliQ water and ethanol, and finally dried.

Methods:

Static contact angles of MilliQ water drop (θw) were measured using Tracker from Teclis instruments. A 0.5µL drop of milliQ water was formed at the end of the needle. The needle was then lowered until the drop contacted the surface and the needle retracted leaving the droplet on the surface. An optical image was then taken, and the contact angle determined within the Windrop software.

XPS spectra were recorded on a ThermoFisher Scientific K-ALPHA spectrometer with a monochromatic AlKα source (hν = 1486.6 eV). 4 different spots of 400 μm^2 are analyzed in each sample. A pressure of 10^{-7} Pa was reached in the chamber. The full spectra (0–1200 eV) were obtained with a constant pass energy of 200 eV and high resolution spectra at a constant pass energy of 40 eV. Charge neutralization was applied during analysis.

The ATR element (Ge, single reflexion) comes from Specac (Fig 2S). ATR-FTIR spectra were obtained on a Nicolet 6700 FT-IR spectrometer (Nicolet Instrument, Madison, WI) equipped with a liquid nitrogen–cooled mercury-cadmium telluride detector (Thermo Fisher Scientific). The spectral resolution was 4 cm^{-1}. Ge crystal was cleaned with Hellmanex 1% solution, then the alkane thiol solution was deposited during one hour. After the solution of alkane thiol was removed and replaced by a 20mM PBS buffer in D_2O (pD 7.0) containing the HSA protein at 1 mg/ml. After 2 h, unadsorbed HSA was washed away twice by buffer exchange. To determine the secondary structure element of each protein, spectra were analyzed with an algorithm based on a second-derivative function and a self-deconvolution procedure (GRAMS and OMNIC software; Thermo Fisher Scientific) to determine the number and wavenumber of individual bands within the spectral range of the amide I band (1700–1600 cm^{-1}).

■ RESULTS AND DISCUSSION

Functionalization of Ge crystal with alkane thiols and characterization of the obtained surfaces and regeneration.

Three different alkane thiol functions with two different chain lengths were used to functionalize Ge (100) plates. Contact angle measurements were used to probe the hydrophilicity or hydrophobicity of the grafted surfaces (see Table 1). The grafting of hydrophobic molecules SAM-CH_3 leads to an increase of the contact angle of water drops by more than 100° as already observed by various authors.[6, 8] Similar values are also reported in the literature for naked Ge, or Ge grafted with hydrophilic SAMs (-OH and -COOH).[5,7]

Table 1: Contact angles of MilliQ water drop (θw) measurements (n=3)

Surfaces	θw
Ge	23°±4°
Ge-S$(CH_2)_{10}CH_3$	107°±2°
Ge-S$(CH_2)_5CH_3$	101°±4°
Ge-S$(CH_2)_{10}$COOH	52°±1°
Ge-S$(CH_2)_5$COOH	59°±1°
Ge-S$(CH_2)_{10}$OH	42°±2°

X-ray photoelectron spectroscopy was carried out to investigate the element composition and oxidation states of the species at the Ge surface before and after each treatment. Figure 1 displays Ge3d, Ge2p and S2p XP spectra for naked Ge surface (black curve), after functionalization by HS-$(CH_2)_5$-CH_3 (red curve), then after regeneration of the Ge surface (blue curve). The green line corresponds to the second grafting, purple line to the tenth grafting and yellow line the regenerated surface after ten cycles of functionalization and regeneration. The bands at 33eV of the Ge3d and at 1220 eV and 1252 eV of the Ge2p are characteristics of the oxide present on the Ge surface.[2, 7] After grafting of SAMs, their intensity decrease due to the modification of the oxide layer on Ge surface. The presence of Ge-S bond is confirmed by the doublet S2p3/2 and S2p1/2 at around 163 eV.[7] The values for Ge-S bond are similar to Au-S bond.[9] The regeneration of the Ge surface was obtained by immersion in Hellmanex solution (1%, incubation 1 hour).

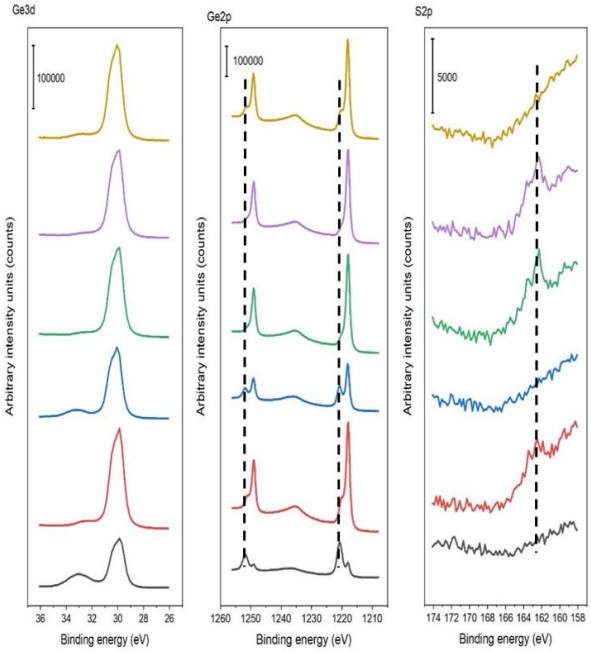


Figure 1: XPS Ge3d (left panel), Ge2p (middle panel) and S2p (right panel) signal of Ge (100) plates surface modified with HS-$(CH_2)_5$-CH_3. Black line corresponds to naked Ge, red line to grafted Ge surface, blue line to regenerated Ge surface (elimination of SAM), green line to the second grafting, purple line to the tenth grafting and yellow line the regenerated surface after ten cycles of functionalization and regeneration.

The XP spectra recorded just after the Ge surface regeneration show the disappearance of the band at 163 eV and the presence of some oxide on the surface of the Ge, as expected for naked Ge surface. The disappearance of the band at 163 eV (S2p) proves the elimination of the SAMs from the Ge surface and its regeneration (Fig. 1. blue curve). The second grafting (green line) or the tenth grafting (purple line) lead to the same modifications as described for the first one.

Ten cycles of functionalization/regeneration of Ge surface were carried out, the XP spectra (Fig. 1, curve yellow) are characteristic of naked Ge with few oxides on its surface, less than before the functionalization. Similarly, functionalization of Ge surface by HS-$(CH_2)_5$-COOH and its regeneration were followed by XPS (See Figure 1S in supplementary material). The same marker bands were observed for the naked Ge surface or functionalized ones. Hellmanex 1% solution is effective for removing SAMs grafted onto the Ge surface regardless of the nature of the SAM: hydrophobic or hydrophilic, charged or not. After ten cycles, the Ge surfaces are regenerated, a slight passivation of the Ge is observed (decrease of the oxide content on the Ge surface).

The grafting of SAMs onto the Ge surface was also checked by polarized ATR-FTIR spectroscopy. Figure 2 displays the ATR-FTIR spectra (*p* polarisation) of the Ge crystal (of a commercial Specac setup) functionalized by alkane thiols (Ge-S-$(CH_2)_{10}CH_3$, Ge-S-$(CH_2)_{10}$COOH and Ge-S-$(CH_2)_{10}$OH) in the range of the C-H stretching modes). The main bands at around 2923 cm^{-1} and 2852 cm^{-1} are assigned to $\nu_{as}CH_2$ and ν_sCH_2 vibrational modes, respectively. The $\nu_{as}CH_3$ (2958 cm^{-1}) and ν_sCH_3 (2872 cm^{-1}) vibrational modes are also observed for the Ge-S-$(CH_2)_{10}CH_3$. The dichroic ratio (A*p* (ν_sCH_2)/ A*s* (ν_sCH_2)) is equal to 1.1 for each SAM, thus the alkyl chains have similar orientation on the Ge surface whatever the head group of the SAMs. This ratio is characteristic of alkyl chains perpendicular to the Ge surface.[10] Similar spectra were recorded for the grafting with shorter alkyl chain length $(CH_2)_5$. The intensity of the bands was lower, as expected for shorter chains (results not shown). Incubation for 1 hour in a 1% Hellmanex solution eliminates grafted thiols on the germanium surface. The measured contact angle is 26°± 5° , in agreement with the value measured for bare Ge (see Table 1). Analysis of the ATR-FTIR spectra also demonstrates the removal of thiols from the surface by Hellmanex. Figure 2 (dotted spectra) shows the disappearance of the all the bands corresponding to the vibrations of the alkyl chains. The process of functionalization and elimination has been performed ten times on the same Ge surface. After 10 functionalizations, expected values of contact angles (θw) for hydrophobic and hydrophilic surfaces are measured, 90° ±4° and 58° ±3° respectively. Similarly, the contact angles value obtained for the naked Ge surface after 10 cycles was 25°±4°, allowing to conclude that the process of functionalization and elimination is robust and does not induce a strong modification of the Ge properties.

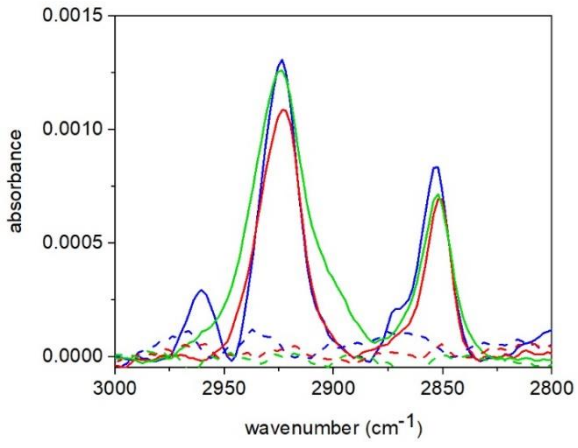


Figure 2: ATR-FTIR spectra of Ge-S-$(CH_2)_{10}CH_3$ (solid blue line), Ge-S-$(CH_2)_{10}$COOH (solid green line), and Ge-S-$(CH_2)_{10}$OH (solid red line), the dotted lines correspond to the ATR-FTIR spectra recorded after the elimination of SAMs by Hellmanex 1% solution.

Secondary structure modification of protein adsorbed on SAMs, monitored by ATR-FTIR spectroscopy on modified Ge crystal. As example, the Human Serum Albumin (HSA) was adsorbed on SAMs with various hydrophilic/hydrophobic properties grafted on Ge crystal and the variation of the secondary structure of the HSA was followed by ATR-FTIR spectroscopy. The presence of the protein on the grafted Ge is proven by the appearance of the amide I and amide II bands at around 1650 cm^{-1} and 1550 cm^{-1} respectively. Similar absorbance were detected for both surfaces hydrophilic or hydrophobic (see Fig 3S in supplementary materials). Figure 3 shows the normalized ATR-FTIR spectra of the HSA in solution (black curve) or adsorbed on Ge modified by HS-$(CH2)_{10}$-OH (red curve) or by HS-$(CH2)_{10}$-CH_3 (blue curve) in the range of amide I and II bands. The profile of the amide I band for the adsorbed HSA is different from the profile of the HSA in solution, indicating a modification of its secondary structure. Table 2 reports the percentage of secondary structure element obtained after the deconvolution and fit process of the amide I bands. Figure 4S (in supplementary materials) shows the fitting of amide I bands. Adsorption on both surfaces induce a large modification of the secondary structure of the HSA. The content of β-sheets and random coil structure increases at the expense of α-helices (-18%). The increase of the β-sheet content is more important on hydrophobic SAM compared to hydrophilic one (+10% and + 5% respectively).

Table 2. Secondary structure of the HSA protein in solution or adsorbed on SAMs deduced from the decomposition of the amide I bands.

Secondary Structure Element	**Wavenumbers** (cm^{-1})	**Percentage of secondary structure element**		
		Solution	$C_{10}OH$	$C_{10}CH_3$
turns	1670	14 ± 1	13 ± 2	14 ± 1
α helix	1653	54 ± 1	36 ± 3	36 ± 1
random	1641	14 ± 1	28 ± 3	22 ± 1
β sheet	1632	18 ± 1	23 ± 2	28 ± 2

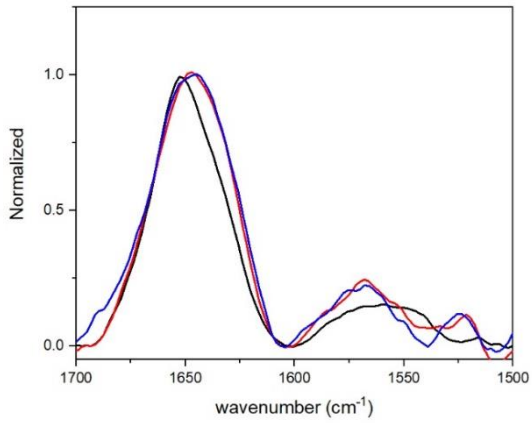


Figure 3: Normalized ATR-FTIR spectra of HSA protein in solution (black curve), adsorbed on Ge-S-$(CH_2)_{10}$ CH_3 (blue curve) or on Ge-S-$(CH_2)_{10}$ OH (red curve)

Sivaramam et al. evaluated by circular dichroism (CD) method the effect of similar alkanethiols (–CH3 and –OH) on the HSA adsorption and they observed the same tendency.[11] Since CD is less sensitive to random structure compared to ATR-FTIR spectroscopy, authors only discuss the α-helix/β-sheet conversion. They observe the decrease of α-helix content due to HSA adsorption on SAMs, and the more important increase of β-sheet content observed on hydrophobic SAM compared to hydrophilic one, which is in good agreement with our observations.

■ CONCLUSION

The SAMs are commonly used to mimic environment of protein or enzyme or modified SAMs to immobilized enzyme. The SAMs are grafted on Au or Ge surfaces *via* thiol function. The interest of grafting SAMs on a germanium surface is that this same surface can be used to determine the organization of the SAMs and the secondary structure of the immobilized protein. In this paper, an innovative simple protocol is described to reversibly graft SAMs on Ge surfaces (plates or commercial ATR setups), which opens the door to easily repeatable experiments and *in-situ* analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

XPS signal of Ge (100) plates surface modified with HS-$(CH_2)_5$-COOH, and after regeneration (10 cycles). ATR-FTIR spectra of HSA protein adsorbed on Ge-S-$(CH_2)_{10}CH_3$ or adsorbed on Ge-S-$(CH_2)_{10}$OH and the deconvolution of the amide I band allowing to determine the % of secondary structural element of the HSA.

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Notes

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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