



HAL
open science

Grafting of Oligo(ethylene glycol)-Functionalized Calix[4]arene-Tetradiazonium Salts for Antifouling Germanium and Gold Surfaces

Pascale Blond, Alice Mattiuzzi, Hennie Valkenier, Ludovic Troian-Gautier, Jean-Francois Bergamini, Thomas Doneux, Erik Goormaghtigh, Vincent Raussens, Ivan Jabin

► To cite this version:

Pascale Blond, Alice Mattiuzzi, Hennie Valkenier, Ludovic Troian-Gautier, Jean-Francois Bergamini, et al. Grafting of Oligo(ethylene glycol)-Functionalized Calix[4]arene-Tetradiazonium Salts for Antifouling Germanium and Gold Surfaces. *Langmuir*, 2018, 34 (21), pp.6021-6027. 10.1021/acs.langmuir.8b00464 . hal-01807882

HAL Id: hal-01807882

<https://univ-rennes.hal.science/hal-01807882v1>

Submitted on 3 Jul 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4
5
6 Grafting of Oligo(ethylene glycol) Functionalized
7
8
9
10 Calix[4]arene-tetra-diazonium Salts for Antifouling
11
12
13
14 Germanium and Gold Surfaces
15
16
17
18
19

20 *Pascale Blond,^{†,‡} Alice Mattiuzzi,^{†,‡} Hennie Valkenier,[⊥] Ludovic Troian-Gautier,[†] Jean-François
21 *Bergamini,[§] Thomas Doneux,^Φ Erik Goormaghtigh,[‡] Vincent Raussens,^{‡,*} and Ivan Jabin^{†,*}**

22
23
24
25 [†] Laboratoire de Chimie Organique, Université Libre de Bruxelles (ULB), avenue F. D. Roosevelt
26 50, CP160/06, B-1050 Brussels, Belgium.
27
28
29

30
31 [‡] Laboratory for the Structure and Function of Biological Membranes, Center for Structural
32 Biology and Bioinformatics, Université Libre de Bruxelles (ULB), avenue F. D. Roosevelt 50,
33 CP206/02, B-1050 Brussels, Belgium.
34
35
36
37

38
39 [⊥] Engineering of Molecular NanoSystems, Ecole Polytechnique de Bruxelles, Université Libre de
40 Bruxelles (ULB), avenue F. D. Roosevelt 50, CP165/64, B-1050 Brussels, Belgium.
41
42
43

44
45 [§] Université de Rennes 1, Institut des Sciences Chimiques de Rennes (Equipe MaCSE), CNRS,
46 UMR 6226, Campus de Beaulieu, Bat 10C, 35042 Rennes Cedex, France.
47
48
49

50
51 ^Φ Chimie Analytique et Chimie des Interfaces, Université Libre de Bruxelles (ULB), Campus de
52 la Plaine, boulevard du Triomphe, CP255, B-1050 Brussels, Belgium.
53
54

55
56 [‡] X4C, Rue Chêne Bonnet 128, 6110 Montigny-le-Tilleul, Belgium.
57
58
59
60

ABSTRACT

Biosensors that can determine protein concentration and structure are highly desired for biomedical applications. For the development of such biosensors, the use of Fourier transformed infra-red (FTIR) spectroscopy with the attenuated internal total reflection (ATR) configuration is particularly attractive but it requires appropriate surface functionalization of the ATR optical element. Indeed, the surface has to specifically interact with a target protein in close contact with the optical element and must display antifouling properties to prevent nonspecific adsorption of other proteins. We here report robust monolayers of calix[4]arenes bearing oEGs chains, which were grafted on germanium and gold surfaces via their tetra-diazonium salts. The formation of monolayers of oEGylated calix[4]arenes was confirmed by AFM, IR and contact angle measurements. The antifouling properties of these modified surfaces were studied by ATR-FTIR spectroscopy and fluorescence microscopy and the non-specific absorption of BSA was found to be reduced by 85% compared to non-modified germanium. In other words, the organic coating by oEGylated calix[4]arenes provides remarkable antifouling properties, opening the way to the design of germanium- or gold-based biosensors.

INTRODUCTION

Proteins are involved in a very large number of biological processes. Even a small change in their concentration, post-translational modifications, or in their native structure can lead to pathologies.¹ Biosensors that enable fast and selective identification and structural characterization

1
2
3 of proteins in complex mixtures represent thus an important target for research.^{2,3} A major
4 challenge in the development of such biosensors is the modification of surfaces by a robust organic
5 monolayer able to specifically interact with a protein⁴ and displaying antifouling properties to
6 prevent nonspecific adsorption phenomena.
7
8
9
10

11
12
13 In this context, there is an increasing interest in the use of germanium-based surfaces because
14 this material can be readily used for Fourier transformed infra-red (FTIR) spectroscopy.^{5,6,7,8} In
15 contrast with other detection methods, FTIR spectroscopy allows to simultaneously collect a
16 wealth of information such as secondary structure and post-translational modifications.^{9,10}
17 Therefore, FTIR spectroscopy with attenuated internal total reflection (ATR) configuration is
18 currently applied to study mono- and multilayers of bioorganic samples.¹¹ Because the evanescent
19 wave propagates outside of the ATR element on a very short distance only, the ATR configuration
20 allows the detection of analytes in aqueous media when they are brought in close contact with the
21 optical element. This ATR element could for example be a Ge crystal with the appropriate
22 geometry. Ge has the advantages over Si (a much more studied element¹²) to have a larger
23 refractive index (resulting in a better signal-to-noise ratio) and to be transparent in a broader
24 spectral range, allowing the study of more important chemical groups absorbing between 1400 and
25 800 cm^{-1} .
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

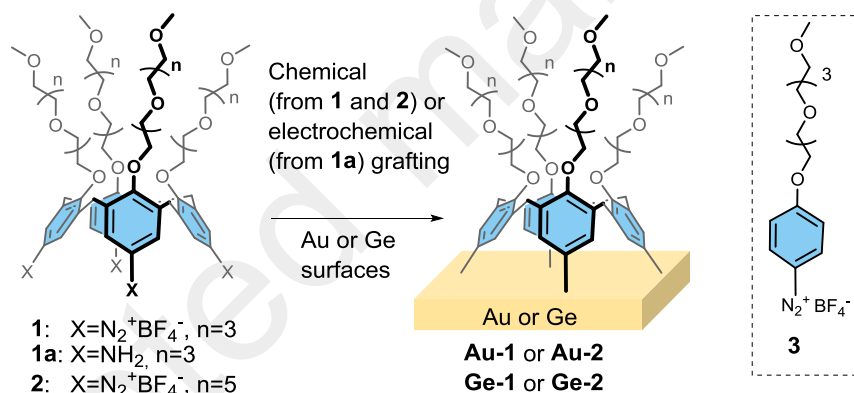
44 Chemisorbed thiols^{6,13} and silane derivatives^{5,8,14,15} are generally used for the chemical
45 modification of Ge surfaces. However, the lack of reproducible grafting protocols and of long-
46 term stability in aqueous media of the obtained monolayers are important limitations of these
47 methods. In contrast, the reductive grafting of aryldiazonium salts^{3,16,17,18} yields stable layers on
48 Ge.^{19,20,21} Nevertheless, this technique generates *in situ* aryl radicals that easily react with the
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 already grafted aryl groups rendering the formation and organization of monolayers difficult to
4
5 control.^{22,23}
6
7

8
9 In this regard, we have recently developed a general strategy for the formation of robust,
10
11 homogeneous and well-organized organic monolayers on various surfaces thanks to the use of
12
13 calix[4]arene-tetra-diazonium salts.^{24,25,26} These compounds can be electrochemically or
14
15 chemically reduced to produce aryl radicals that can be grafted *via* multiple covalent bonds. The
16
17 structure and geometry of the calix[4]arenes do not allow any additional grafting, preventing the
18
19 formation of multilayers. Furthermore, when grafting calix[4]arenes functionalized at the small
20
21 rim with for instance appending COOH groups, the resulting calixarene layer can be further post-
22
23 functionalized under mild conditions by various chemical species.²⁴ An additional feature, in
24
25 contrast to all the other known methods, is the preparation of binary monolayers of controlled
26
27 composition in a single step from the grafting of binary mixtures of calix[4]arene-tetra-diazonium
28
29 salts.^{27,28}
30
31
32
33
34

35
36 Regarding the nonspecific adsorption of proteins and biomacromolecules, this is typically
37
38 prevented by surface modification with small shielding molecules or polymers. Coatings based on
39
40 oligo(ethylene glycol) (oEG), polysaccharides, zwitterionic compounds or perfluoroalkyls are
41
42 some examples.^{29,30,31,32,33,34} As oEGs are nontoxic and nonimmunogenic, they are widely used in
43
44 medical applications.^{35,36,37,38} The dominant way through which proteins adhere to surfaces is by
45
46 hydrophobic effect.^{39,40} Uncharged oEGylated surfaces interact with water molecules, preventing
47
48 this hydrophobic effect and thus effectively shielding the surface from protein fouling. It is
49
50 noteworthy that the grafting of antifouling coatings on Ge has been scarcely reported.^{14,41}
51
52
53
54
55
56
57
58
59
60

In order to develop a new ATR device suitable for biosensing applications, we have investigated the grafting on Ge of a robust monolayer of calix[4]arenes decorated with multiple oEGs arms. For this, calix[4]arene-tetra-diazonium salts bearing oEGs substituents of different lengths (**1** and **2**) and a reference compound **3**⁴² were synthesized (Scheme 1). The feasibility of the grafting was first evaluated on standard gold surfaces through previously developed electrochemical and chemical grafting processes.²⁶ As Ge is a semi-conducting surface, the conventional electrochemical grafting process is not appropriate and thus only the chemical process was then used. Finally, the antifouling properties of the surfaces that were modified with oEGylated calixarenes were evaluated by FTIR spectroscopy and confocal laser scanning fluorescence microscopy upon exposure to proteins.



Scheme 1. Chemical grafting of calix[4]arenes **1** and **2** or electrochemical grafting of **1a**. Inset: structure of reference diazonium salt **3**. Note that the representation of the grafted calixarene does not necessarily imply that its four aryl units are linked to the surface.

EXPERIMENTAL SECTION

1
2
3 **Chemicals and materials.** All solvents and reagents were at least of reagent grade quality and
4 were purchased either from Alfa Aesar, Sigma-Aldrich, TCI, Roth or Acros organics. All reactions
5 were performed under an inert atmosphere. Reactions were magnetically stirred and monitored by
6 thin layer chromatography using Merck-Kieselgel 60F254 plates. Flash chromatography was
7 performed with silica gel 60 (particle size 35-70 μm) supplied by Merck. Anhydrous DMF was
8 obtained from Acros organics. Anhydrous THF was obtained from distillation on
9 Na/benzophenone. Ultrapure water was obtained via a Millipore Milli-Q system (18.2 M Ω cm).
10 The fluorescent FITC-BSA (fluorescein isothiocyanate BSA conjugate) was purchased from
11 Sigma-Aldrich as well as the BSA used for ATR-FTIR spectroscopy experiments. Gold-coated
12 silicon wafer (1000 Å layer thickness) was purchased from Sigma-Aldrich. Both sides polished
13 germanium squares (10 x 10 x 0.5 mm) and germanium single-crystal triangular prisms (base 6.8
14 mm x 45 mm length and an internal incident angle of 45°) were purchased from ACM (France).
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29

30 **Caution!** Although we have not encountered any problem, it is noted that diazonium salts
31 derivatives are potentially explosive and should be handled with appropriate precautions.
32
33

34 Both calix[4]arene-tetra-oEGs **1** and **2** were prepared following the same strategy. The detailed
35 synthesis of compound **1** (Scheme 2) is given here, while details for the compounds **2** and **3** are
36 reported in the SI.
37
38
39
40
41

42 **15,35,55,75-tetra-tert-butyl-12,32,52,72-tetrakis(2-(2-methoxyethoxy)ethoxy)-1,3,5,7(1,3)-**
43 **tetrabenzenacyclooctaphane 6.** Commercial *p*-^tBu-calix[4]arene (2.0 g, 3.1 mmol) was dissolved
44 in dry DMF (50 mL). NaH (60% dispersion in oil, 0.840 g, 21.0 mmol) and 2,5,8,11-
45 tetraoxatridecan-13-yl 4-methylbenzenesulfonate **4** (7.6 g, 21.0 mmol) were added and the reaction
46 mixture was stirred for 24 hours at 75°C under inert atmosphere. The reaction mixture was then
47 brought to room temperature and quenched dropwise with 5 mL of HCl (0.5 M). The mixture was
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 then concentrated under reduced pressure. The resulting oil was dissolved in CH₂Cl₂ (200 mL),
4 the organic layer was washed with H₂O (3 x 150 mL) and the combined aqueous layers were
5 extracted with CH₂Cl₂ (300 mL). The combined organic layers were concentrated under reduced
6 pressure. The crude oil was purified by flash chromatography on silica gel (EtOAc/MeOH =
7 85:15). The oil was washed with H₂O to yield the compound **6** as a yellow oil (4.2 g, 3.0 mmol,
8 96%).

9
10
11
12
13
14
15
16
17 ¹H NMR (300 MHz, CDCl₃, 298K), δ (ppm): 1.06 (s, 36H), 3.09 (d, *J* = 12 Hz, 4H), 3.37 (s,
18 12H), 3.49-3.71 (m, 48H), 3.93 (t, *J* = 4.5 Hz, 8H), 4.09 (t, *J* = 4.5 Hz, 8H), 4.41 (d, *J* = 12 Hz,
19 4H), 6.75 (s, 8H). ¹³C NMR (100 MHz, CDCl₃, 298K), δ (ppm): 31.2, 31.6, 33.9, 59.2, 70.5, 70.6,
20 70.6, 70.7, 70.8, 70.8, 72.1, 73.0, 125.1, 133.9, 144.7, 153.4. FTIR, ν (cm⁻¹): 3412, 2952, 2921,
21 2899, 2867, 1644, 1480 1457, 1392, 1361, 1300, 1249, 1199, 1107, 1062, 1027, 944, 869. HRMS:
22 calcd. for C₈₀H₁₂₈O₂₀ (M+H)⁺ 1409.91 found 1409.91.

23
24
25
26
27
28
29
30
31 **15,35,55,75-tetra-nitro-12,32,52,72-tetrakis(2-(2-methoxyethoxy)ethoxy)-1,3,5,7(1,3)-**
32 **tetrabenzenacyclooctaphane 8**. Note that compound **8** was already described in the literature⁴³
33 but is here prepared *via* another synthetic strategy. Calix[4]arene-tetra-oEG₄ **6** (4.2 g, 3.0 mmol)
34 was dissolved in CH₂Cl₂ (100 mL). A mixture of glacial CH₃COOH/fuming HNO₃ (1:1) (22 mL)
35 was added at 0°C and the reaction mixture was stirred for 5 hours at room temperature. The
36 reaction mixture was concentrated under reduced pressure. The resulting oil was dissolved in
37 CH₂Cl₂ (100 mL), the organic layer was washed with H₂O (3 x 70 mL) and the combined aqueous
38 layers were extracted with CH₂Cl₂ (150 mL). The combined organic layers were concentrated
39 under reduced pressure. The crude oil was purified by flash chromatography on silica gel
40 (DCM/Acetone = 65:35) to yield compound **8** as a yellow oil (2.5 g, 1.8 mmol, 61%).
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

¹H NMR spectrum of compound **8** is in accordance with the one reported in the literature.⁴³ ¹H NMR (300 MHz, CDCl₃, 298K), δ (ppm): 3.33-3.44 (m, 16H), 3.48-3.63 (m, 48H), 3.80 (t, *J* = 4.5 Hz, 8H), 4.24 (t, *J* = 4.5 Hz, 8H), 4.65 (d, *J* = 12 Hz, 4H), 7.58 (s, 8H).

12,32,52,72-tetrakis(2-(2-methoxyethoxy)ethoxy)-1,3,5,7(1,3)-tetrabenzenacyclooctaphane-15,35,55,75-tetraamine 1a. Note that compound **1a** was already described in the literature⁴³ but is here prepared *via* another synthetic strategy. Calix[4]arene-tetra-NO₂-tetra-oEG₄ **8** (0.901 g, 0.660 mmol) and Pd/C (53 mg, 0.498 mmol) were suspended in EtOH (18 mL). Hydrazine hydrate (3 mL, 61.8 mmol) was added dropwise and the reaction mixture was stirred for 15 hours at reflux. The reaction mixture was filtered on Celite and the Celite was washed with EtOH and CH₂Cl₂. The filtrate was concentrated under reduced pressure to yield the compound **1a** as a yellow oil (0.798 g, 0.641 mmol, 97%).

¹H NMR spectrum of compound **1a** is in accordance with the one reported in the literature.⁴³ ¹H NMR (300 MHz, CD₃OD, 298K), δ (ppm): 2.94 (d, *J* = 13 Hz, 4H), 3.35 (s, 12H), 3.48-3.70 (m, 48H), 3.89 (t, *J* = 5 Hz, 8H), 4.03 (t, *J* = 5 Hz, 8H), 4.40 (d, *J* = 13 Hz, 4H), 6.17 (s, 8H).

12,32,52,72-tetrakis(2-(2-methoxyethoxy)ethoxy)-1,3,5,7(1,3)-tetrabenzenacyclooctaphane-15,35,55,75-tetrakis(diazonium) 1. Calix[4]-tetra-aniline-tetra-oEG₄ **1a** (50 mg, 0.040 mmol) was solubilized in dry acetonitrile (0.5 mL). At -40°C, NOBF₄ (46 mg, 0.400 mmol) was added and the reaction mixture was stirred for 2 hours at -40°C under inert atmosphere. The reaction mixture was concentrated under reduced pressure at room temperature to yield the compound **1** as a yellow/orange oil. Due to the well-known low stability of diazonium groups, compound **1** was used without further purification for the grafting experiments.

HRMS analysis was not performed because of the low stability of compound **1** against temperature.

¹H NMR (600 MHz, CD₃CN, 298K), δ (ppm): 3.30 (s, 12H), 3.43-3.59 (m, 48H), 3.78 (d, *J* = 14 Hz, 4H), 3.84 (br t, 8H), 4.49 (br t, 8H), 4.72 (d, *J* = 14 Hz, 4H), 8.02 (br s, 8H). ¹³C NMR (150 MHz, CD₃CN, 298K), δ (ppm): 31.0, 58.9, 70.4, 70.7, 70.7, 70.9, 71.0, 71.0, 72.5, 77.5, 107.7, 135.0, 138.7, 168.7. FTIR, ν (cm⁻¹): 3083, 2942, 2883, 2267, 1571, 1443, 1355, 1293, 1264, 1096, 1025, 922, 846.

Electrochemical grafting strategy. Electrochemical measurements were conducted in a three-electrode cell using a Ag|AgCl saturated KCl electrode as reference electrode, a large area platinum foil as counter electrode, and a polycrystalline gold disk (1.6 mm in diameter, from Bioanalytical Systems) as working electrode, all connected to an Autolab PGSTAT30 (Metrohm Autolab) potentiostat equipped with a ScanGen module.

Gold electrodes were first polished with alumina paste (1 μm particles) and sonicated in ultrapure water for 10 minutes before being dried under a flux of nitrogen. The diazonium salt **1** was prepared *in situ* in 0.5 M aqueous HCl in the presence of NaNO₂ (8 eq.) from the corresponding calix[4]-tetra-aniline **1a** (1.5 mM). The gold substrate was functionalized through the reduction of the *in situ* generated diazonium cations by six voltammetric cycles, the potential being swept between +0.5 V and -0.4 V versus Ag|AgCl electrode. Once the grafting was achieved, the surfaces were thoroughly rinsed with ultrapure water and sonication was applied for 10 minutes.

Chemical grafting strategy. Caution! Piranha solution is a very strong oxidant and should be handled very carefully. Gold surfaces were first immersed in a piranha solution (H₂SO₄/H₂O₂ 3:1) for 10 minutes and sonicated for 5 minutes. They were subsequently washed with concentrated sulphuric acid and with ultrapure water and then dried under argon atmosphere. Germanium surfaces were first immersed in ultrapure water and then in ethanol; sonication was applied each

1
2
3 time for 5 minutes. They were then rinsed with Et₂O and dried under argon atmosphere. The
4
5 surfaces were dipped in a 5 mM solution of the diazonium salt (**1-3**) in aqueous 0.1 M sodium
6
7 hydroxide for 2 hours, without stirring in order to avoid any mechanical damage of the surface by
8
9 the magnetic stirrer bar (a minimal volume of solution is used). Once the grafting was achieved,
10
11 all the surfaces were thoroughly washed with ultrapure water and then acetonitrile. Sonication was
12
13 applied each time for 5 minutes. The surfaces were then dried under argon flow.
14
15

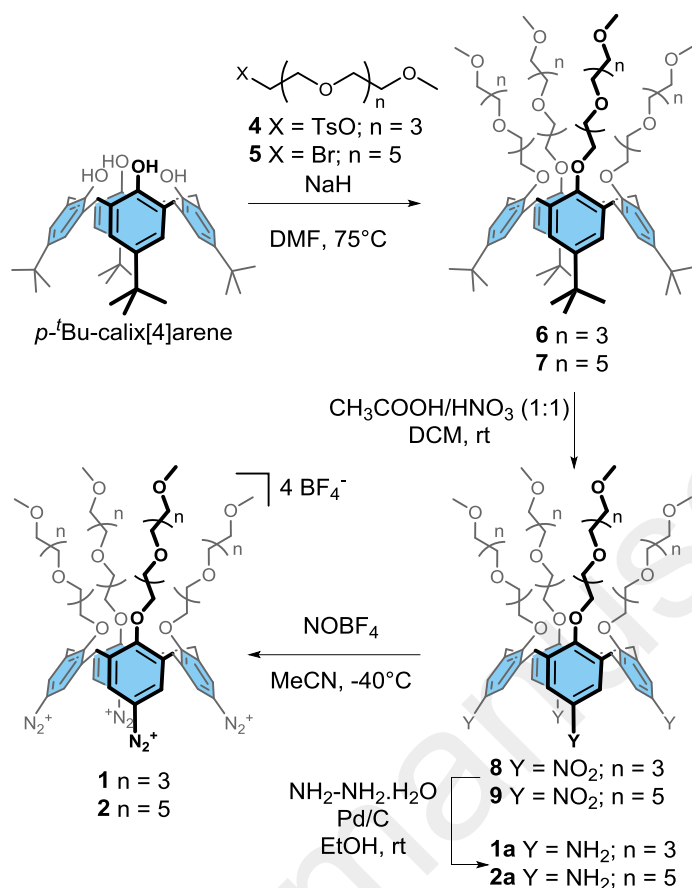
16
17 **ATR-FTIR** spectra were recorded at 22°C on a Bruker Equinox 55 FTIR spectrophotometer
18
19 equipped with a liquid nitrogen-cooled mercury-cadmium-telluride (MCT) detector. The
20
21 spectrophotometer was continuously purged with dried air. The target chemicals were deposited
22
23 in solution on a germanium single-crystal internal reflection element (triangular prism of 6.8 x 45
24
25 mm, with an internal incidence angle of 45°) and the solvent was removed with a flow of nitrogen
26
27 gas. The spectra from the grafted monolayer were obtained following the chemical grafting of the
28
29 calix[4]arene target directly on the germanium internal reflection element. In each case, bare
30
31 germanium was used for the background spectrum. The nonspecific adsorption experiment was
32
33 measured in a flow-through cuvette with a 100 µg/mL solution of BSA in phosphate buffer media
34
35 in D₂O (PBS-D₂O) and a flow rate of 10 µL/min. Opus software (4.2.37) was used to record 128
36
37 scans with a nominal resolution of 2 cm⁻¹ for Figures 1 and S54 and 64 scans with a nominal
38
39 resolution of 4 cm⁻¹ for Figures 2, S55 and S56. Data were processed and analysed using the home-
40
41 written Kinetics package in Matlab R2013a by subtraction of water vapor, baseline correction,
42
43 apodization at 16 cm⁻¹.
44
45
46
47
48

49 **Confocal Laser Scanning Fluorescence Microscopy:** fluorescence images were recorded with
50
51 a Nikon Ti-Eclipse inverted microscope, equipped with a laser emitting at 488 nm. The emitted
52
53 light was collected in the epi-fluorescence mode and a dichroic mirror was employed to select
54
55
56
57
58
59
60

1
2
3 wavelengths shorter than 540 nm. A bandpass emission filter (515–530 nm), matching well the
4 emission spectrum of fluorescein, was placed in front of the photo-multiplier tube. A 10×
5 magnification objective (NA = 0.30, working distance 16 mm) was employed. The images were
6 processed with the software ImageJ.
7
8
9
10
11
12
13
14

15 RESULTS AND DISCUSSION

16
17
18 Both calix[4]arene-tetra-oEGs **1** and **2** were prepared following the strategy developed for other
19 calix[4]arene-tetra-diazonium salts (Scheme 2).^{24,26} First, a tetra O-alkylation of *p*-^tBu-
20 calix[4]arene constrained the macrocycle in a cone conformation and allowed the introduction of
21 the oEGs arms on the small rim. Then, a sequence of ipso-nitration / reduction afforded the tetra-
22 anilines **1a** and **2a** in respectively 57 % and 52 % overall yields from *p*-^tBu-calix[4]arene. Finally,
23 a diazotation reaction yielded target diazonium salts **1** and **2** whose structures were clearly
24 confirmed by 1D and 2D NMR spectroscopy. Grafting experiments were performed on gold and
25 germanium surfaces with both calix[4]arenes **1** and **2** as well as with the reference compound **3** for
26 comparison (Scheme 1).
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Scheme 2. Synthesis of calix[4]arene-tetra-oEG_n-tetra-diazonium salts **1** and **2**.

Similarly to previously reported calixarenes,²⁶ grafting of the new oEGylated calixarenes was first evaluated on a gold substrate, using calix[4]-tetra-aniline precursor **1a**, from which the corresponding tetra-diazonium salt **1** was prepared *in situ* by adding an excess of NaNO₂ in acidic conditions. Reduction of the diazonium cations was performed by six voltammetric cycles. The density of the electrografted layer was characterized by analyzing its blocking property towards the electrochemical response of the redox probe Fe(CN)₆^{3-/4-}.²⁴ Complete inhibition of the electrochemical response was found, indicating that a dense organic layer had been formed at the electrode surface (Figure S51).

1
2
3 Having found that the electrochemical grafting methodology was applicable to the newly
4 synthesized calix[4]arene-tetra-oEGs, we subsequently undertook the chemical grafting of the
5 diazonium salts (**1-3**) on Au and Ge substrates. The previously reported protocols for the grafting
6 of aryl diazonium salts on Ge in organic solvents requires an oxide-free Ge surface obtained by
7 treatment with an aqueous HF solution.^{19,20,21} Here, the grafting procedure on Ge substrate was
8 performed in a basic aqueous solution and involved the *in situ* formation of diazoates.^{44,45,46} Under
9 these aqueous conditions, the laborious and hazardous pre-treatment with aqueous HF is not
10 needed, as the Ge oxide at the surface is water soluble.⁴⁷ The chemical grafting of diazonium salts
11 **1, 2** and **3** was performed upon immersion of the Au and Ge substrates in an aqueous 0.1 M NaOH
12 solution containing 5 mM of the desired diazonium salt for 2 hours. This procedure afforded gold
13 surfaces **Au-1**, **Au-2** and **Au-3** and germanium surfaces **Ge-1** and **Ge-2** (Scheme 1). Each grafting
14 experiment was repeated at least three times to assess the reproducibility.

15
16
17 All these modified surfaces were then characterized by contact angle measurements and the
18 results were compared to those obtained with bare surfaces (*i.e.*, surfaces that were treated
19 similarly to the modified ones but in the absence of the diazonium salt). Contact angles of $62 \pm 9^\circ$
20 and $30 \pm 10^\circ$ were obtained respectively for bare Au and Ge substrates (Table 1). For all the
21 modified oEGylated surfaces, the contact angle was found to be *ca.* 56° . These results clearly
22 confirm the presence of the oEG layer on all the modified surfaces and highlight the reproducibility
23 of the grafting process. Furthermore, the value of *ca.* 56° is in agreement with those found in
24 literature for oEGylated surfaces.⁴⁸

25
26
27 **Table 1.** Contact angle measurements and thickness estimations of the grafted organic layers.

Contact angle ^a (°)	Thickness ^b (nm)
--------------------------------	-----------------------------

Bare Au ^c	62 ± 9	-
Au-1	55 ± 5	2.4 ± 0.4
Au-2	56 ± 4	n.d. ^d
Au-3	56 ± 7	19.0 ± 1.0
Bare Ge ^c	30 ± 10	-
Ge-1	56 ± 1	3.0 ± 0.3
Ge-2	56 ± 3	n.d. ^d

^a Average values obtained by multiple analyses repeated on several surfaces. ^b Determined by AFM measurement (see text). ^c The bare surfaces were treated similarly to the modified ones but in the absence of the diazonium salt. ^d n.d.: not determined.

Atomic Force Microscopy (AFM) measurements were performed on the surfaces **Au-1**, **Au-3**, **Ge-1** and on the bare gold and germanium substrates. All the modified surfaces exhibited a surface topography similar to that of the corresponding bare substrate, indicating a thin uniform deposit on the surface (Figure S52 and S53). Next, thicknesses of the organic layers were estimated in contact mode through scratching experiments. The AFM tip was used to scratch a square area on the modified samples by exercising a force sufficient to remove the organic part without damaging the substrate. Using the height difference between both areas of the surface profile (Figure S52 and S53), thicknesses of the organic layer of 2.4 and 3.0 nm were found for **Au-1** and **Ge-1** respectively (Table 1). These values correspond well with the height of the calix[4]arene-tetra-oEG₄ structure (*ca.* 2.2 nm estimated from MM2 energy minimizations with ChemBio3D software), suggesting that a monolayer of grafted calix[4]arene-tetra-oEGs **1** was obtained on gold and germanium. In the case of the control surface **Au-3**, a thickness of 19.0 nm was estimated, clearly indicating the formation of multilayers. This result highlights the crucial role played by the calixarene structure in the formation of monolayers.

The germanium surfaces **Ge-1** and **Ge-2** were then analyzed by ATR-FTIR spectroscopy. For comparison, the IR absorbance spectra of compounds **1**, **2** and their corresponding anilines **1a** and **2a** were recorded (Figures 1 and S54). Typical bands belonging to the calix[4]arene core and oEG groups were observed both for the compounds and the corresponding monolayers. The asymmetric COC stretching from the oEG chains around 1100 cm^{-1} is clearly visible in all spectra. Other bands can be attributed to the calix[4]arene moieties such as the symmetric COC_{Ar} stretching around 1050 – 1020 cm^{-1} and the aromatic ring stretching around 1460 cm^{-1} . The IR results thus clearly confirm the grafting of calixarenes **1** and **2** on Ge.

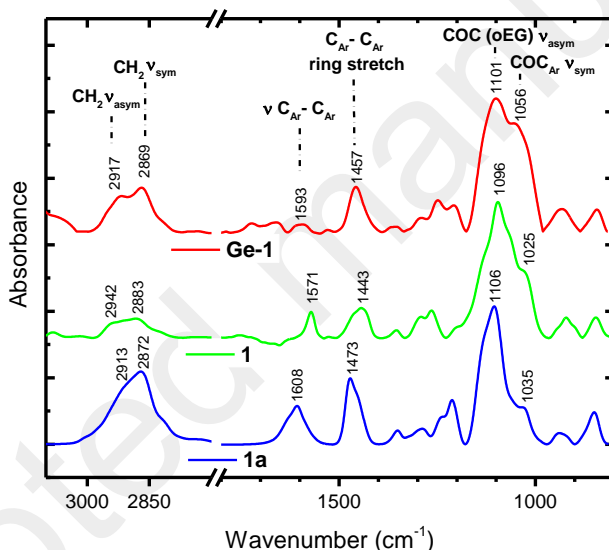


Figure 1. ATR-FTIR absorption spectra from 3100-2700 and 1800-800 cm^{-1} of modified surface **Ge-1** (red), calix[4]arene-tetra-diazonium salt **1** (green) and calix[4]-tetra-aniline **1a** (blue).

The nonspecific adsorption of proteins on bare Ge and on oEGylated Ge substrates was monitored by ATR-FTIR spectroscopy. Surfaces **Ge-1** and **Ge-2** were compared to evaluate the influence of the chain length of oEGs on the protein adsorption. A flow-through cuvette with a

1
2
3 solution of bovine serum albumin (BSA, 100 $\mu\text{g/mL}$) in phosphate buffer media in D_2O (PBS-
4 D_2O) at 22°C was used to analyze the nonspecific binding of BSA on the surfaces. BSA is used as
5
6 model protein in many investigations because it is one of the most abundant proteins found in
7
8 blood and it adheres very well to surfaces. First, the stability of the calixarene layer under the study
9
10 conditions was evaluated through exposure of the surface **Ge-1** to a flow (10 $\mu\text{L}/\text{min}$) of PBS- D_2O
11
12 buffer during 16 h. The decomposition of the organic layer should give rise to the appearance of
13
14 negative absorbance bands in the regions where the calixarene signals are located (*e.g.*, at 1100
15
16 cm^{-1}). This was not observed, indicating that the calix[4]arene monolayer is stable under aqueous
17
18 conditions for at least 16 hours (Figure S55). The surfaces **Ge-1** and **Ge-2** were then placed under
19
20 a flow (10 $\mu\text{L}/\text{min}$) of the PBS- D_2O medium for 10 min in order to reach an equilibrium state
21
22 under aqueous conditions. The flow was then switched to the solution of BSA in PBS- D_2O for 16
23
24 hours and finally back to PBS- D_2O for 5 hours. Infrared spectra were recorded every minute for
25
26 the first 20 minutes and then every 10 minutes till the end of the experiment. Adsorbed BSA was
27
28 characterized by two bands whose intensities were increasing over time: the amide-I' at 1640 cm^{-1} ,
29
30 which is mainly associated with the C=O(ND) stretching vibrations and the amide-II' at 1450
31
32 cm^{-1} , which results mainly from in plane ND bending vibrations (Figure 2a and S56). The integral
33
34 of the amide-I' band at 1640 cm^{-1} was calculated for each spectrum. The graph shows that the
35
36 binding of BSA to surfaces **Ge-1** and **Ge-2** is reduced by more than 85% in comparison to bare Ge
37
38 (Figure 2b). No difference was found between **Ge-1** and **Ge-2**, showing that an oEG length of four
39
40 on the calix[4]arenes is sufficient for the effective prevention of nonspecific protein adsorption on
41
42 Ge. Such an efficiency in the reduction of nonspecific adsorption has been reported scarcely and,
43
44 in the case of the previous systems, either a very thick coverage was necessary (*e.g.*, polymer
45
46 brushes)^{48,49} or a limited stability was observed.³⁶
47
48
49
50
51
52
53
54
55
56
57
58
59
60

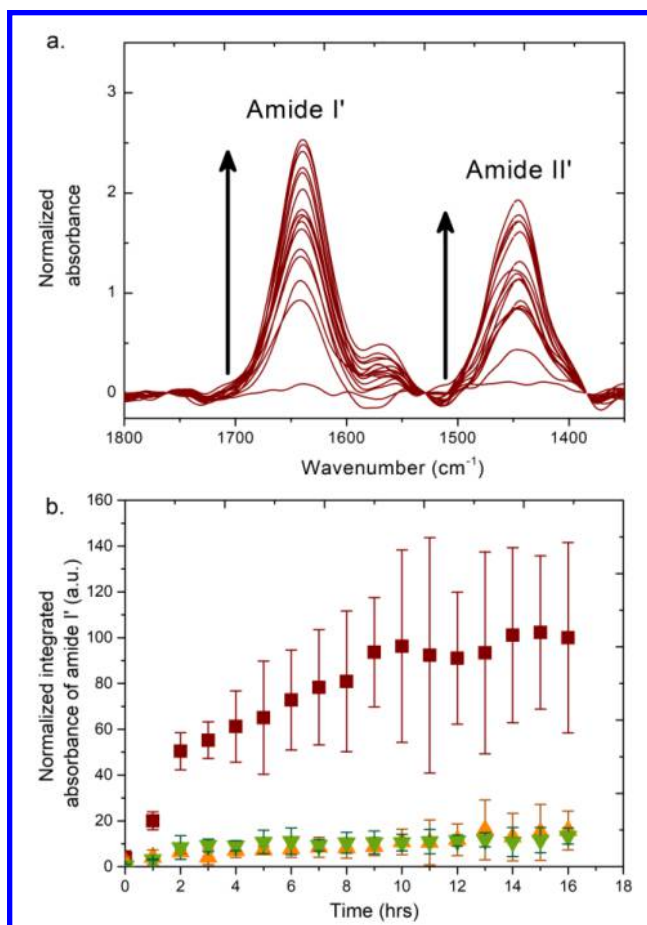


Figure 2. (a) ATR-FTIR absorption spectra from 1800-1350 cm^{-1} reported for every hour during the BSA nonspecific adsorption experiment on bare Ge. (b) Normalized adsorption of BSA on bare Ge substrate (dark red \blacksquare), Ge-1 (orange \blacktriangle) and Ge-2 (green \blacktriangledown). The error bars correspond to the standard deviation obtained from two or three independent measurements.

The nonspecific adsorption of proteins was also evaluated by confocal laser scanning fluorescence microscopy. A defined area of Au and Ge surfaces was first modified with the calix[4]arene-tetra-oEG₄ **1**, the surfaces were then submerged in a 100 $\mu\text{g}/\text{mL}$ solution of fluorescent FITC-BSA in PBS buffer for 6 hours, followed by rinsing in PBS buffer for 16 hours. The resulting fluorescence images are shown in Figure 3. On both images, two areas can be clearly

distinguished: the unmodified one serving as control (top) and the one modified with the calix[4]arene (bottom). On the modified zone, the fluorescence is significantly decreased, showing a drastic reduction of the nonspecific adsorption of BSA. This result is not quantitative, but gives a good indication of the remarkable antifouling character of the modified surfaces.

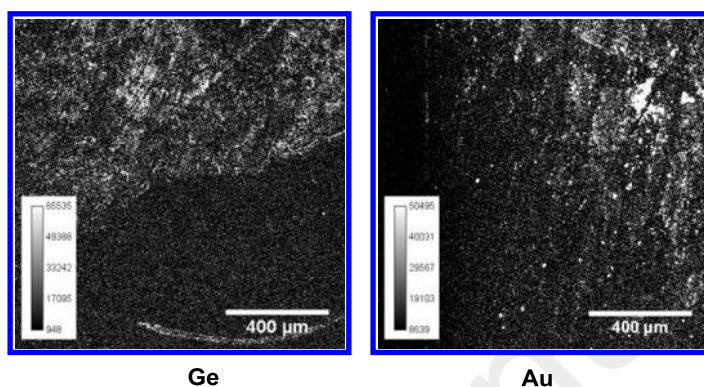


Figure 3. Fluorescence images acquired on gold and germanium surfaces that were partially modified with the calix[4]arene-tetra-oEG₄ **1** and subsequently immersed in a 100 μg/mL solution of fluorescently labelled BSA in PBS buffer for 6 hours.

CONCLUSIONS

In conclusion, we have developed a reproducible procedure for the grafting of thin and robust organic monolayers of calix[4]arene-tetra-oEGs on Ge and Au that do not require any laborious or dangerous pre-treatment. The significant antifouling properties of these monolayers were demonstrated. The nonspecific adsorption of proteins was decreased by more than 85% compared to bare Ge. Such an efficiency is unique if we consider the thickness (*ca.* 3 nm) and the high stability of the calixarene layer. The modified Ge surfaces thus constitute promising devices for the development of ATR-FTIR-biosensors. Current developments in our laboratories are directed

1
2
3 toward the grafting of calix[4]arene-tetra-oEGs with terminal post-functionalizable groups (*e.g.*
4 COOH, azide or alkyne groups) that can serve for the controlled immobilization of proteins.
5
6
7
8
9
10

11 ASSOCIATED CONTENT

12 13 14 15 **Supporting Information**

16
17
18 The Supporting Information is available free of charge on the ACS Publications website at DOI:.

19
20
21 Detailed procedure for the preparation and characterization of the synthesized compounds, NMR
22 spectra, AFM results, and additional FTIR experiments. This material is available free of charge
23 via the Internet at <http://pubs.acs.org>.
24
25
26
27
28
29
30

31 AUTHOR INFORMATION

32 33 34 **Corresponding Authors**

35
36
37 *E-mail: ijabin@ulb.ac.be; vrauss@ulb.ac.be
38
39

40 **ORCID**

41
42 Pascale Blond: 0000-0001-8515-1737

43
44 Alice Mattiuzzi: 0000-0002-3784-8449

45
46 Hennie Valkenier: 0000-0002-4409-0154

47
48 Ludovic Troian-Gautier: 0000-0002-7690-1361

49
50 Thomas Doneux: 0000-0002-9082-8826

51
52 Erik Goormaghtigh: 0000-0002-2071-2262
53
54
55
56
57
58
59
60

Vincent Raussens: 0000-0002-7507-1845

Ivan Jabin: 0000-0003-2493-2497

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The “Actions de Recherches Concertées” of the Fédération Wallonie-Bruxelles and the ULB (Ph.D. grant to P.B.) are acknowledged for financial support.

REFERENCES

-
- ¹ Gestwicki, J. E.; Garza, D. Chapter 10 - Protein Quality Control in Neurodegenerative Disease. In *Progress in Molecular Biology and Translational Science*, Teplow, D. B., Ed. Academic Press: **2012**, Vol. 107, pp 327-353.
- ² Leech, D. Affinity Biosensors. *Chem. Soc. Rev.* **1994**, *23*, 861-878.
- ³ Cao, C.; Zhang, Y.; Jiang, C.; Qi, M.; Liu, G. Advances on Aryldiazonium Salt Chemistry Based Interfacial Fabrication for Sensing Applications. *ACS Appl. Mater. Inter.* **2017**, *9*, 5031-5049.
- ⁴ Camarero, J. A. New Developments for the Site-Specific Attachment of Protein to Surfaces. *Biophys. Rev. Lett.* **2006**, *01*, 1-28.

1
2
3
4
5 ⁵ Devouge, S.; Conti, J.; Goldsztein, A.; Gosselin, E.; Brans, A.; Voue, M.; De Coninck, J.;
6
7 Homble, F.; Goormaghtigh, E.; Marchand-Brynaert, J. Surface Functionalization of Germanium
8
9 ATR Devices for Use in FTIR-Biosensors. *J. Colloid Interf. Sci.* **2009**, *332*, 408-415.

10
11
12
13 ⁶ Schartner, J.; Gavriljuk, K.; Nabers, A.; Weide, P.; Muhler, M.; Gerwert, K.; Kotting, C.
14
15 Immobilization of Proteins in their Physiological Active State at Functionalized Thiol Monolayers
16
17 on ATR-Germanium Crystals. *Chembiochem* **2014**, *15*, 2529-2534.

18
19
20
21 ⁷ Goldzstein, A.; Aamouche, A.; Homblé, F.; Voué, M.; Conti, J.; De Coninck, J.; Devouge, S.;
22
23 Marchand-Brynaert, J.; Goormaghtigh, E. Ligand–Receptor Interactions in Complex Media: A
24
25 New Type of Biosensors for the Detection of Coagulation Factor VIII. *Biosens. Bioelectron.* **2009**,
26
27 *24*, 1831-1836.

28
29
30
31 ⁸ Schartner, J.; Nabers, A.; Budde, B.; Lange, J.; Hoeck, N.; Wiltfang, J.; Kötting, C.; Gerwert,
32
33 K. An ATR–FTIR Sensor Unraveling the Drug Intervention of Methylene Blue, Congo Red, and
34
35 Berberine on Human Tau and A β . *ACS Med. Chem. Lett.* **2017**, *8*, 710-714.

36
37
38
39 ⁹ Goormaghtigh, E.; Ruyschaert, J.-M.; Raussens, V. Evaluation of the Information Content in
40
41 Infrared Spectra for Protein Secondary Structure Determination. *Biophys. J.* **2006**, *90*, 2946-2957.

42
43
44
45 ¹⁰ Derenne, A.; Goormaghtigh, E. FTIR Spectroscopy as a Multi-Parameter Analytical Tool.
46
47 *Biopharm. Int.* **2017**, *30*, 35-40.

48
49
50
51 ¹¹ Dole, M. N.; Patel, P. A.; Sawant, S. D.; Shedpure, P. S. *Advance Applications of Fourier*
52
53 *Transform Infrared Spectroscopy* **2011**, Vol. 7, p 159-166.

1
2
3
4
5 ¹² Ammar, M.; Smadja, C.; Ly, G. T.; Tandjigora, D.; Vigneron, J.; Etcheberry, A.; Taverna, M.;
6
7 Dufour-Gergam, E. Chemical Engineering of Self-Assembled Alzheimer's Peptide on a Silanized
8
9 Silicon Surface. *Langmuir* **2014**, *30*, 5863-5872.

10
11
12
13 ¹³ Holmberg, V. C.; Rasch, M. R.; Korgel, B. A. PEGylation of Carboxylic Acid-Functionalized
14
15 Germanium Nanowires. *Langmuir* **2010**, *26*, 14241-14246.

16
17
18 ¹⁴ Nabers, A.; Ollesch, J.; Schartner, J.; Kotting, C.; Genius, J.; Haussmann, U.; Klafki, H.;
19
20 Wiltfang, J.; Gerwert, K. An Infrared Sensor Analysing Label-Free the Secondary Structure of the
21
22 Abeta Peptide in Presence of Complex Fluids. *J. Biophotonics* **2016**, *9*, 224-234.

23
24
25
26 ¹⁵ Voué, M.; Goormaghtigh, E.; Homble, F.; Marchand-Brynaert, J.; Conti, J.; Devouge, S.; De
27
28 Coninck, J. Biochemical Interaction Analysis on ATR Devices: a Wet Chemistry Approach for
29
30 Surface Functionalization. *Langmuir* **2007**, *23*, 949-955.

31
32
33
34 ¹⁶ Pinson, J.; Podvorica, F. Attachment of Organic Layers to Conductive or Semiconductive
35
36 Surfaces by Reduction of Diazonium Salts. *Chem. Soc. Rev.* **2005**, *34*, 429-439.

37
38
39 ¹⁷ Pinson, J. Aryl Diazonium Salts: New Coupling Agents in Polymer and Surface Science.
40
41 Chehimi, M. M. *Wiley-VCH: Weinheim* **2012**, pp 1-35.

42
43
44
45 ¹⁸ Delamar, M.; Hitmi, R.; Pinson, J.; Saveant, J. M. Covalent Modification of Carbon Surfaces
46
47 by Grafting of Functionalized Aryl Radicals Produced from Electrochemical Reduction of
48
49 Diazonium Salts. *J. Am. Chem. Soc.* **1992**, *114*, 5883-5884.

50
51
52
53 ¹⁹ Collins, G.; Fleming, P.; O'Dwyer, C.; Morris, M. A.; Holmes, J. D. Organic Functionalization
54
55 of Germanium Nanowires using Arenediazonium Salts. *Chem. Mater.* **2011**, *23*, 1883-1891.

1
2
3
4
5 ²⁰ Girard, A.; Geneste, F.; Coulon, N.; Cardinaud, C.; Mohammed-Brahim, T. SiGe
6 Derivatization by Spontaneous Reduction of Aryl Diazonium Salts. *Appl. Surf. Sci.* **2013**, *282*,
7 146-155.
8
9

10
11
12 ²¹ Lefevre, X.; Segut, O.; Jegou, P.; Palacin, S.; Joussetme, B. Towards Organic Film Passivation
13 of Germanium Wafers Using Diazonium Salts: Mechanism and Ambient Stability. *Chem. Sci.*
14 **2012**, *3*, 1662-1671.
15
16
17
18

19
20 ²² Combellas, C.; Jiang, D.-e.; Kanoufi, F.; Pinson, J.; Podvorica, F. I. Steric Effects in the
21 Reaction of Aryl Radicals on Surfaces. *Langmuir* **2009**, *25*, 286-293.
22
23
24

25
26 ²³ Combellas, C.; Kanoufi, F.; Pinson, J.; Podvorica, F. I. Sterically Hindered Diazonium Salts
27 for the Grafting of a Monolayer on Metals. *J. Am. Chem. Soc.* **2008**, *130*, 8576-8577.
28
29

30
31 ²⁴ Mattiuzzi, A.; Jabin, I.; Mangeney, C.; Roux, C.; Reinaud, O.; Santos, L.; Bergamini, J. F.;
32 Hapiot, P.; Lagrost, C. Electrografting of Calix[4]arene Diazonium Salts to Form Versatile Robust
33 Platforms for Spatially Controlled Surface Functionalization. *Nat. Commun.* **2012**, *3*, 1130-1138.
34
35
36
37

38
39 ²⁵ Troian-Gautier, L.; Valkenier, H.; Mattiuzzi, A.; Jabin, I.; den Brande, N. V.; Mele, B. V.;
40 Hubert, J.; Reniers, F.; Bruylants, G.; Lagrost, C.; Leroux, Y. Extremely Robust and Post-
41 Functionalizable Gold Nanoparticles Coated with Calix[4]arenes via Metal-Carbon Bonds. *Chem.*
42 *Commun.* **2016**, *52*, 10493-10497.
43
44
45
46

47
48
49 ²⁶ Troian-Gautier, L.; Martínez-Tong, D. E.; Hubert, J.; Reniers, F.; Sferrazza, M.; Mattiuzzi,
50 A.; Lagrost, C.; Jabin, I. Controlled Modification of Polymer Surfaces through Grafting of
51 Calix[4]arene-Tetradiazoate Salts. *J. Phys. Chem. C* **2016**, *120*, 22936-22945.
52
53
54
55
56
57
58
59
60

1
2
3
4
5 ²⁷ Santos, L.; Mattiuzzi, A.; Jabin, I.; Vandencastele, N.; Reniers, F.; Reinaud, O.; Hapiot, P.;
6
7 Lhenry, S.; Leroux, Y.; Lagrost, C. One-Pot Electrografting of Mixed Monolayers with Controlled
8
9 Composition. *J. Phys. Chem. C* **2014**, *118*, 15919-15928.

10
11
12 ²⁸ Valkenier, H.; Malytskyi, V.; Blond, P.; Retout, M.; Mattiuzzi, A.; Goole, J.; Raussens, V.;
13
14 Jabin, I.; Bruylants, G. Controlled Functionalization of Gold Nanoparticles with Mixtures of
15
16 Calix[4]arenes Revealed by Infrared Spectroscopy. *Langmuir* **2017**, *33*, 8253-8260.

17
18
19
20 ²⁹ Yam, C. M.; Deluge, M.; Tang, D.; Kumar, A.; Cai, C. Preparation, Characterization,
21
22 Resistance to Protein Adsorption, and Specific Avidin–Biotin Binding of Poly(amidoamine)
23
24 Dendrimers Functionalized with Oligo(ethylene glycol) on Gold. *J. Colloid Interf. Sci.* **2006**, *296*,
25
26 118-130.

27
28
29
30 ³⁰ Banerjee, I.; Pangule, R. C.; Kane, R. S. Antifouling Coatings: Recent Developments in the
31
32 Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. *Adv.*
33
34 *Mater.* **2011**, *23*, 690-718.

35
36
37
38 ³¹ Ederth, T.; Ekblad, T.; Pettitt, M. E.; Conlan, S. L.; Du, C.-X.; Callow, M. E.; Callow, J. A.;
39
40 Mutton, R.; Clare, A. S.; D'Souza, F.; Donnelly, G.; Bruin, A.; Willemsen, P. R.; Su, X. J.; Wang,
41
42 S.; Zhao, Q.; Hederos, M.; Konradsson, P.; Liedberg, B. Resistance of Galactoside-Terminated
43
44 Alkanethiol Self-Assembled Monolayers to Marine Fouling Organisms. *ACS Appl. Mater. Inter.*
45
46 **2011**, *3*, 3890-3901.

47
48
49
50 ³² Kitano, H.; Kawasaki, A.; Kawasaki, H.; Morokoshi, S. Resistance of Zwitterionic Telomers
51
52 Accumulated on Metal Surfaces Against Nonspecific Adsorption of Proteins. *J. Colloid Interf. Sci.*
53
54 **2005**, *282*, 340-348.

³³ Brady, R. F.; Aronson, C. L. Elastomeric Fluorinated Polyurethane Coatings for Nontoxic Fouling Control. *Biofouling* **2003**, *19*, 59-62.

³⁴ Li, S.; Yang, D.; Tu, H.; Deng, H.; Du, D.; Zhang, A. Protein Adsorption and Cell Adhesion Controlled by the Surface Chemistry of Binary Perfluoroalkyl/Oligo(ethylene glycol) Self-Assembled Monolayers. *J. Colloid Interf. Sci.* **2013**, *402*, 284-290.

³⁵ Harris, J. M. Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications. *Plenum Press: New York* **1992**.

³⁶ Comenge, J.; Puentes, V. F. The Role of PEG Conformation in Mixed Layers: from Protein Corona Substrate to Steric Stabilization Avoiding Protein Adsorption. *ScienceOpen Research* **2015**, DOI: 10.14293/S2199-1006.1.SOR-MATSCI.A0Z6OM.v1.

³⁷ Ozcelik, B.; Ho, K. K. K.; Glattauer, V.; Willcox, M.; Kumar, N.; Thissen, H. Poly(ethylene glycol)-Based Coatings Combining Low-Biofouling and Quorum-Sensing Inhibiting Properties to Reduce Bacterial Colonization. *ACS Biomater. Sci. Eng.* **2017**, *3*, 78-87.

³⁸ Riedel, T.; Riedelová-Reicheltoová, Z.; Májek, P.; Rodriguez-Emmenegger, C.; Houska, M.; Dyr, J. E.; Brynda, E. Complete Identification of Proteins Responsible for Human Blood Plasma Fouling on Poly(ethylene glycol)-Based Surfaces. *Langmuir* **2013**, *29*, 3388-3397.

³⁹ Brash, J. L.; Horbett, T. A. Proteins at Interfaces. In *Proteins at Interfaces II*, American Chemical Society: **1995**, Vol. 602, pp 1-23.

⁴⁰ Awadhiya, A.; Tyeb, S.; Rathore, K.; Verma, V. Agarose Bioplastic-Based Drug Delivery System for Surgical and Wound Dressings. *Eng. Life Sci.* **2016**, *17*, 204-214.

1
2
3
4
5 ⁴¹ Schartner, J.; Güldenhaupt, J.; Mei, B.; Rögner, M.; Muhler, M.; Gerwert, K.; Kötting, C.
6
7 Universal Method for Protein Immobilization on Chemically Functionalized Germanium
8 Investigated by ATR-FTIR Difference Spectroscopy. *J. Am. Chem. Soc.* **2013**, *135*, 4079-4087.
9

10
11
12 ⁴² Fairman, C.; Ginges, J. Z.; Lowe, S. B.; Gooding, J. J. Protein Resistance of Surfaces Modified
13 with Oligo(ethylene glycol) Aryl Diazonium Derivatives. *ChemPhysChem* **2013**, *14*, 2183-2189.
14

15
16
17 ⁴³ Zhao, Y.; Ryu, E.-H. Solvent-Tunable Binding of Hydrophilic and Hydrophobic Guests by
18 Amphiphilic Molecular Baskets. *J. Org. Chem.* **2005**, *70*, 7585-7591.
19

20
21
22
23 ⁴⁴ Griffete, N.; Herbst, F.; Pinson, J.; Ammar, S.; Mangeney, C. Preparation of Water-Soluble
24 Magnetic Nanocrystals Using Aryl Diazonium Salt Chemistry. *J. Am. Chem. Soc.* **2011**, *133*, 1646-
25
26
27 1649.
28

29
30
31 ⁴⁵ Doyle, C. D.; Rocha, J.-D. R.; Weisman, R. B.; Tour, J. M. Structure-Dependent Reactivity
32 of Semiconducting Single-Walled Carbon Nanotubes with Benzenediazonium Salts. *J. Am. Chem.*
33
34
35 *Soc.* **2008**, *130*, 6795-6800.
36

37
38
39 ⁴⁶ Podvorica, F. I.; Kanoufi, F.; Pinson, J.; Combellas, C. Spontaneous Grafting of Diazoates on
40 Metals. *Electrochim. Acta* **2009**, *54*, 2164-2170.
41

42
43
44 ⁴⁷ Amy, S. R.; Chabal, Y. J. Passivation and Characterization of Germanium Surfaces. In
45 Advanced Gate Stacks for High-Mobility Semiconductors, Dimoulas, A.; Gusev, E.; McIntyre, P.
46
47
48 C.; Heyns, M., Eds. *Springer Berlin Heidelberg: Berlin, Heidelberg*, **2007**, pp 73-113.
49

50
51
52 ⁴⁸ Chen, Y. W.; Chang, Y.; Lee, R. H.; Li, W. T.; Chinnathambi, A.; Alharbi, S. A.; Hsiue, G.
53
54
55 H. Adjustable Bioadhesive Control of PEGylated Hyperbranch Brushes on Polystyrene Microplate
56
57
58 Interface for the Improved Sensitivity of Human Blood Typing. *Langmuir* **2014**, *30*, 9139-9146.
59

1
2
3
4
5 ⁴⁹ Demirci, S.; Kinali-Demirci, S.; Jiang, S. A Switchable Polymer Brush System for Antifouling
6 and Controlled Detection. *Chem. Commun.* **2017**, *53*, 3713-3716.
7
8
9

10
11
12
13
14
15
16
17 **TOC Graphic**
18

