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3 Interactions between elastin-like peptides (ELPs) and insulating poly ortho-aminophenol (PoAP)
4 membrane investigated by AFM and XPS
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Abstract

The present investigation was undertaken to explore the mutual recognition of the pentapeptide (ValGlyGlyValGly)_n, a hydrophobic elastin-like peptide (ELP), suspended in deionized water in monomer (n=1) and trimer (n=3) forms, and the outer surface of a very thin, insulating polymer, PoAP, electrochemically grown on a platinum foil by Cyclic Voltammetry, CV, in neutral media (PBS, I=0.1M), immersed in the suspension.

As a prior task, the proved propensity of the ValGlyGlyValGly sequence, at the given minimal length (n-repeats ≥ 3), to self-assembly into amyloid-like fibrils when solubilized in aqueous environment, was here considered in the prospect of testing PoAP surfaces for the specific detection of amyloid precursors.

Based on our knowledge of chemical structure and physical properties of both bio-macromolecule families, gained in previous studies, we have focused our attention on the efficacy of binding sites offered to ELP fibrils by PoAP in its 'as prepared' form or properly modified either by post-synthesis oxidation and by adsorption/entrapping of ELP monomer(s) with or without 'protecting' terminal groups.

Consistently with all ways of preparation, the best surfaces, recognizable by the trimer fibrils, are those modified to carry a larger number of carbonyls, particularly, by entrapment of ELP monomer(s) during PoAP electro-synthesis, using an imprinting- inspired methodology.

The degree of attachment of fibrillar aggregates, detected by AFM and XPS techniques, provide unequivocal evidence of the cooperative forces involving PoAP-ELPs interactions. Interesting results have emerged all converging in the perspective of qualifying the proposed Pt/PoAP/ELP systems as bio-detectors to improve diagnostic exploitation in Alzheimer Disease.

Keywords

XPS, AFM, Poly ortho-Amino Phenol (PoAP), Elastin-like peptides (ELPs), Amyloids, Peptides-imprinted CV-polymerization

INTRODUCTION

One important marker of Alzheimer's disease (AD) is the deposition and accumulation of amyloid (A β) fibrils into insoluble plaques being associated to age-dependent cognitive changes and dementia [1-8]. The A β peptides, generated by the amyloidogenic cleavage of Amyloid Precursor Protein (APP) [5,6, 9-11], are highly predisposed to self-aggregate and to transform, rapidly, if over-produced, into β -stranded oligomers and proto-fibrils finally evolving into amyloids accumulating around neurons. It is widely recognized that of these A β variants, soluble monomers/oligomers are the most abundant forms in preclinical stages, while their level drops with disease onset and progression due to their sinking into amyloid plaques. It is thus of great importance the development of biosensors for the specific determination of amyloid-beta peptides and their aggregates, considering either early and late stage detection as well as total A β variants. As recently reported [5, 12-19], in addition to the very few protocols already approved for clinical diagnosis, multi detection systems based on aptamers and molecularly imprinted polymers working in body fluids other than CSF are expected to emerge in the near future.

In particular, among the currently proposed biosensors, those holding adsorbed or 'imprinted' receptors are used to an increasing extent for selectively monitoring peptides and proteins. Therefore, the 'anchoring' of hydrophobic and strongly self-aggregating amyloid peptides represent new challenge in the diagnostics of amyloid precursors [19-24].

As a natural evolution of the research work dedicated by our group to the synthesis and characterization of nanostructured biopolymers and to their bio-analytical applications, the present contribution proposes a model system based on the conjugated action of polymers and peptides as an analytical tool for a deeper understanding of the mutual interactions of amyloidogenic peptides, to improve their diagnostic exploitation.

Within this context, we recall the *CV* electro-synthesis of ortho-aminophenol (oAP), a very interesting organic compound due to its ability to form conductive or insulating polymers, PoAP, under electrochemical oxidation in acidic and neutral/basic media, respectively [25-29]. Moreover, we have synthesized, as innovative compounds, self-assembling hydrophobic elastin-like polypeptides (ELPs), of the general type (XxxGlyGlyZzzGly)_n (Xxx, Zzz=Val, Leu), that have been extensively characterized [30-34], and tested for biocompatibility with mouse 3T3 fibroblasts [35,36].

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3 Among PoAP and ELP samples, we have in turn selected insulating PoAP and the ELP sequence
4 ValGlyGlyValGly (reported hereinafter as VGGVG), as the most suitable compounds for the
5 present application.
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8 Briefly, insulating PoAP was revealed by AFM investigation to be a homogeneous membrane, fully
9 adhering to the underneath platinum, easy to handle, with a constant repeat formula, as derived by
10 XPS, made of alternating quinoneimine and oAP units and water molecules firmly retained within
11 the polymer structure. Reproducibility tests have shown PoAP to be highly stable even under ultra
12 high vacuum (UHV); thus, most likely, strong H-bonds occur along the polymer chains, perhaps
13 necessary for PoAP alignment. We could extrapolate that, sustained by water molecules, the short
14 PoAP chains (estimated 10 nm, on average) are aligned vertically, ending with imino groups
15 progressively transforming to 'carbonyls' by post-synthesis oxidation in air [25,27, 37,38].
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18 The ELP sequence (VGGVG)_n was selected due to its high propensity to self-assemble in amyloid-
19 like fibrils when suspended in water at a given length ($n \geq 3$) [32,35,36] and being already studied
20 in variable repeat forms, solvent choices and operative conditions: as a monomer ($n=1$) with and
21 without protecting hindering terminal groups, as a trimer ($n=3$) and as a log-normal dispersed
22 polymer chains (with the degree of polymerization, n , centered on 5). Modeling of the water-
23 induced self-assembly was also attempted for poly (VGGVG) in comparison with the homologue
24 poly (LGGVG) [31] based on the cooperative reinforcement of carbonyls H-bonds and hydrophobic
25 interactions by aliphatic chains of their extended β -sheet structures, revealed by FTIR studies. Thus,
26 to the specific aim of providing suitable bio-receptors for amyloids and their precursors, knowing
27 that all amyloid fibrils share a common self-assembly pathway and final β -pleated sheet structure
28 [39-42], these amyloidogenic elastin-like peptides were sought to reproduce, properly, the
29 molecular events of A β variants as indicators of AD biomarkers.
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32 Based on the two branches of results, we have then planned experiments in the order herein
33 reported, following the procedures listed in the experimental section:
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- 36 a) XPS and AFM studies of VGGVG pentapeptides and (VGGVG)₃ pentadecapeptide (trimer)
37 deposited on silicon substrate from water suspensions, as integrative of previous
38 investigation on ELPs self-assembly [30-34], carried under identical modalities;
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- 40 b) Study of the direct interaction between insulating PoAP, freshly prepared or stored in air for
41 days before use, and (VGGVG)₃ trimer, long aged in deionized water;
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3 c) Study of PoAP and (VGGVG)₃ interactions, mediated by VGGVG monomers pre-adsorbed
4 on PoAP surfaces from water solutions, differently aged at ambient conditions, monitoring
5 the influence of VGGVG terminal groups.
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8 d) Study of PoAP and (VGGVG)₃ interactions, mediated by VGGVG monomers entrapped at
9 or near the PoAP surface, during CV electro-synthesis, finding inspiration on Molecularly
10 Imprinted Polymer (MIP) technology [43-45], more precisely, on bi-dimensional MIP [46]
11 given the ultrathin thickness of PoAP membrane adhering on platinum, estimated to be less
12 than 10nm [25-27, 37,38]
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17 Regarding PoAP, we have focused our attention on the binding propensity of the outermost
18 carbonyls, and on their concentration and efficacy as a function of the storage time.
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21 Regarding ELP sequences, we have given the priority to both unprotected and terminal-protected
22 VGGVG monomers, i.e. to the pentapeptide unity, known to be too short in length for evolving as
23 amyloid fibrils, likely suitable to be used as surface receptors for the trimer fibrils, if properly
24 adsorbed/entrapped on PoAP. Previous studies have amply demonstrated that amyloid fibrils
25 formed by (VGGVG)₃ are much smaller and more homogeneously distributed when deposited on
26 substrates, compared to those formed by poly(VGGVG) [30-34]. The minor tendency to associate
27 and entangle with each other leaves the trimer, at the concentration (0.1mg/mL) used in this work,
28 to self-assembly in a kind of pre-fibrillar state: therefore, the old-aged (VGGVG)₃ suspension,
29 available from previous experiments [35,36] was considered suitable for our scope.
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37 Regarding the layered Pt/PoAP/ELP systems, attention was posed on the action of VGGVG
38 monomers as surface bio-detectors, exploiting two methods for their immobilization onto the
39 polymeric film, by surface adsorption and by physical entrapping in the same manner as usually
40 done for the two-dimensional MIP. *It is known, indeed, that these peptide sequences tend to
41 spontaneously identify similar repetition units, making then a more complex structure, the repeated
42 extended β -sheet, by the formation of inter-chain hydrogen bonds, reinforced by hydrophobic
43 interactions [30,39,40].*
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48 In order to understand their assembly and their performance as amyloids detectors, the systems
49 were all tested by XPS and AFM, before and after immersion in the aged suspension of trimer
50 fibrils, also considering the influence of the underneath platinum.
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MATERIALS AND METHODS

Chemicals

The monomer of ortho-aminophenol, oAP, (Fluka, purity 99%, FW = 109.13 g/mol) was purified, before being used, by recrystallization in ethyl acetate (Sigma Aldrich, purity 99%, d = 0.9 g/mL, bp = 76.5 °C) and with activated carbon, as suggested by Ortega [47]. Monomer oAP solutions were prepared in supporting electrolyte just before their use. The procedures for the synthesis of unprotected VGGVG and Boc-VGGVG-OEt pentapeptides and for the pentadecapeptide (VGGVG)₃ are reported in details in previous studies [34-36, 48]. Pure water supplied by Milli-Q RG unit from Millipore (Bedford, MA, USA) was used throughout. All other chemicals were of analytical grade and were used without further purification.

Experimental setup

Electrochemical apparatus and film deposition

Electrochemical experiments were carried out with an EG&G PAR model 263A potentiostat/galvanostat. Data acquisition and potentiostat control were accomplished with a desktop computer running the M270 Electrochemical Research Software (EG&G), version 4.23. All experiments were carried out at room temperature in a standard three-electrode cell employing an Ag/AgCl/KCl (sat) reference electrode and a platinum counter electrode. The working electrode was a platinum foil (10×15×0.127mm, 99.99%, Aldrich). No action was taken to remove oxygen from solutions. Platinum working electrodes were cleaned following the reported procedure [27]. The insulating PoAP film was electrosynthesized by cyclic voltammetry, scanning the electrode potential for 20 cycles between -0.1 and +0.9 V (vs. Ag/AgCl, saturated KCl) in a 5 mM oAP solution in phosphate buffer (I=0.1 M, pH 7) at a scan rate of 50 mV/s.

All the electrochemically synthesized PoAP films were then washed with double-distilled water and dried at room temperature in nitrogen atmosphere before their ex-situ analyses or further manipulations.

ELPs absorption onto PoAP/Pt electrodes

The PoAP/Pt modified electrodes were dipped into ELPs (VGGVG or Boc-VGGVG-OEt) solution, 0.1 mg/mL, for 6 hours at 37°C, subsequently rinsed with Milli-Q water and left to dry overnight before proceeding with AFM and XPS analyses.

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3 All the Pt/PoAP/ELP electrodes were then washed with double-distilled water and dried at room
4 temperature in nitrogen atmosphere before their ex-situ analyses or further manipulations.
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6 7 *ELPs entrapment onto PoAP/Pt electrodes*

8 The modified Pt/PoAP/ELP electrode was obtained by cyclic voltammetry (CV), scanning the
9 electrode potential for just one cycle between -0.1 and +0.9 V (vs. Ag/AgCl, saturated KCl) in a 5
10 mM oAP solution in phosphate buffer (I=0.1 M, pH 7) at a scan rate of 50 mV/s. After the addition
11 of 500 μ L of the 0.1 mg/mL VGGVG (or Boc-VGGVG-OEt) solution to the supporting electrolyte
12 (8 mL), the PoAP electropolymerization has been prolonged for other 19 voltammetric scans.
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17 In order to check the sensing capabilities of these systems, the modified electrodes were dipped into
18 (VGGVG)₃ suspension for 6 hours at 37°C, subsequently rinsed with Milli-Q water and left to dry
19 overnight before proceeding with AFM and XPS analyses.
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22 23 *XPS measurements*

24 XPS analysis were performed by a Phoibos 100-MCD5 spectrometer, operating in Medium Area
25 lens mode (spot of $\varnothing = 2$ mm, entrance slit of 7x20 mm). Spectra were acquired with achromatic Al
26 K α radiation (1486.6 eV) operating at 10 kV and 10 mA. The pressure in the analysis chamber was
27 typically about 10^{-9} mbar during acquisition. A pass energy of 9 eV was used to collect the wide
28 and the detailed spectra, using fixed analyzer transmission (FAT) operation mode with channel
29 widths of 1.0 and 0.1 eV, respectively. All XP spectra were acquired at a 90° take-off angle. The
30 energy scale of the spectrometer was calibrated with Cu2p_{3/2} (932,7 eV) and Au4f_{7/2} (84,0 eV)
31 signals using pure metals (Johnson Matthey) for spectroscopic analysis.
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38 39 *Curve-fitting procedure*

40 The XPS spectra were analysed using a curve-fitting program “Googly” which gives to each
41 individual peak its own intrinsic Shirley-like background and extrinsic tail, as fully described in
42 previous works [49,50]. Peak areas were converted to atomic composition using established
43 procedures and the appropriate sensitivity factors, SF [39]. The criteria adopted for data elaboration
44 were based on preliminary analyses of reference compounds and literature data, also available
45 online [51], to assure the correct sample stoichiometry, in the limit of XPS accuracy [52,53].
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48 The energy scales of the detailed regions are converted to binding energy so as to facilitate
49 comparison of the curve fitted results with literature data and the peak assignments, reported in the
50 relevant tables, are corrected for surface charging by referring to C1s aromatic carbon, used as an
51 internal standard and set at 284.8 eV.
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AFM analysis

AFM images were carried out by using the XE-120 microscope (Park Systems) in air and at room temperature. Data acquisition was carried out in non-contact mode at scan rates between 0.15 and 2.20 Hz, using rectangular Si cantilevers (NCHR, Park Systems, tip radius less than 5 nm) with the nominal resonance frequency and force constant of 330 kHz and 42 N/m, respectively.

RESULTS AND DISCUSSION

a) XPS and AFM analyses of reference bio-macromolecules

AFM images and XPS curve-fitted spectra of three ELPs, H₂-VGGVG-OH, Boc-VGGVG-OEt and (VGGVG)₃ deposited on silicon wafer from water suspension are shown in Figures 1 and 2. XPS curve-fitting results of Figure 2 are summarized in Table 1.

These surface analyses complement those related to protected Boc-VGGVG-OEt characterized in the solid state [34] and insulating PoAP, fully characterized in previous works [25-27, 37,38], allowing a more comprehensive interpretation of data.

For instance, the secondary structures of unprotected H₂-VGGVG-OH were already assigned by circular dichroism (CD) and nuclear magnetic resonance (NMR) investigations [54] and interpreted as an incipient tendency of this pentapeptide to 'head to tail' interactions, when it is present in pure water in the ionized H₃⁺-VGGVG-O⁻ state. Present data support previous observations demonstrating that deposits from water suspension show a more ordered aggregation compared to its protected homologous (see also supplementary materials).

Moreover, previous XPS analyses on powdered Boc-VGGVG-OEt revealed intra-chain C=O...H-N< bonds responsible for the folded secondary structure of this terminal-protected pentapeptide [34]. Similar XPS results and globular (amorphous) aggregation were obtained investigating peptide deposits on silicon from water suspension, as reported in Figures 1b, 2b.

Regarding the amyloidogenic (VGGVG)₃ (Figures 1c, 2c), XPS reveals the carbonyl splitting due to the action of bridging water, associated to amyloid fibrils as reported for poly VGGVG [30-31, 34]. AFM images unequivocally show the presence of amyloid-like fibrils, shorter and thinner with respect to those formed by poly VGGVG [30,31] and more homogeneously distributed (coexisting with pre-fibrillar aggregates of regular morphology).

The morphology of the polymer PoAP [25,27], instead, is totally deprived of structures. As already shown, insulating PoAP is reproducibly electro-synthesized by CV, in neutral media, with an average thickness of about 10 nm, as estimated by both AFM and XPS, as a thin film perfectly adhering to the platinum substrate. Its growth is self-limited by the fast current decay when the

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3 substrate coverage is complete. The curve-fitting of XPS spectra, acquired at different take-off
4 angles, gives highly reproducible results concerning the chemical formula derived as repeat unit, the
5 presence of water within the polymer chains and of carbonyls on the outermost layers, increasing
6 with time by post-synthesis oxidation of terminal C=N groups [26].
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9 Based on previously reported PoAP specificities and indications on ELPs self-assembly, the use of
10 the insulating PoAP-ELPs system, for each combination shown in the following paragraphs, was
11 designed to deepen the understanding of the aggregation phenomena underlying the incipient
12 formation of amyloids, using XPS and AFM as combined means of investigation. Results of XPS
13 curve-fitted spectra, selected as appropriate and indicative of all experiments performed, are cited
14 along the text and summarized in [Table 2](#).
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21 *b) Study of the direct interaction of insulating PoAP, freshly prepared or stored in air for days
22 before use, and (VGGVG)₃ aged in deionized water.*
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24 To perform this study, insulating PoAP was first electro-synthesized by CV on platinum, as
25 previously defined, then the rinsed rectangular shaped Pt/PoAP electrode was immersed in a long
26 aged (VGGVG)₃ suspension for 6 hours at 37°C, using the same interaction time and temperature of
27 previous investigation on 3T3 fibroblasts' adhesion onto ELPs-coated surfaces [35,36].
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31 Figures 3 compare the magnified AFM images and C1s spectra of Pt/PoAP before and after the
32 exposure to (VGGVG)₃ suspension. It is clearly evident that both analyses reveal little interaction
33 between (VGGVG)₃ and freshly prepared PoAP although XPS seems to indicate, by the change of
34 the C1s peak shape, the adsorption of very thin layers not distinguishable by AFM from the
35 substrates' morphologies.
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40 However, knowing the steady increase of carbonyls on PoAP surface due to oxidation of C=N
41 groups in air, the same experiment was repeated using the Pt/PoAP electrode left in ambient
42 conditions for 15 days, after preparation. Figures 4 and Table 2 clearly show different results at this
43 time point. The AFM image (4d) reveals the presence of aggregates, referable to trimer pseudo-
44 fibrils, locally massed on the aged PoAP surface. XPS curve-fitted spectra (4a-c), difficult to
45 accurately quantify because of the uneven morphology/topography, qualitatively confirms the
46 appearance of additional signals, if compared to the curve-fitted C1s, O1s and N1s regions of the
47 "as prepared" PoAP [25, 26], clearly related to trimer adsorption on PoAP. Focusing on C1s, the
48 most significant XPS region, we can identify these signals, being labeled in Figure 4a, but absent in
49 both the C1s spectra of Figures 5, related to Pt/PoAP surfaces, differently aged, before immersion
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3 into (VGGVG)₃ suspension. Indeed, C1s spectra of freshly prepared, Figure 5a, and in air-stored,
4 Figure 5b, PoAP, show the same chemical groups and differences in peak shapes due to the increase
5 of carbonyl contents, **passing from 7.0 to 10.6 At%, respectively, as derived from Table 2.**

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7 Thus, the effect of air oxidation on the external groups seems quite beneficial for our purposes.
8 Since the increase of carbonyl amount causes a greater trimer adsorption on PoAP, it can be
9 supposed that their interaction is mediated by PoAP carbonyls, as terminal functionalities, likely
10 through H-bonds with the ELPs NH-C=O peptide groups, the same inter-chain bonds of amyloid
11 peptides. **The surface reconstruction of PoAP due to ageing, influencing its activity towards fibrils
12 adsorption, cannot be disregarded a priori, however, topographical/conformational changes are
13 hardly discernable in AFM images of Figures 4c,d related to fresh and aged PoAP, respectively.**

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15 Therefore, we can only assume that carbonyls present on the outermost surface, attributed to *a*
16 *posteriori* oxidation of imino groups, are numerically inadequate on freshly prepared PoAP to bond
17 (VGGVG)₃ efficiently, while, the prolonged PoAP storage in air increases their abundance allowing
18 a better anchoring along the trimer sequence.

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20 However, this method is time-requiring and storing conditions could be difficult for PoAP to be
21 reproduced. Therefore, in order to functionalize in a more systematic way our Pt/PoAP electrode,
22 we tried to pre-adsorb on it the shorter peptide sequence, VGGVG. **It was expected that the right
23 monomer(s) coverage would facilitate a more copious and homogeneous (VGGVG)₃ adsorption on
24 PoAP, having the same functional groups and the same amino acid sequence of its repeat.**

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35 *c) Study of PoAP and (VGGVG)₃ interactions mediated by VGGVG monomers pre-adsorbed*
36 *on PoAP surfaces from water solutions, differently aged, in air at room temperature*
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40 To pre-adsorb VGGVG monomers on Pt/PoAP surface the procedure adopted for the trimer
41 detection was used, as reported in the experimental section.

42 Since we have demonstrated that unprotected and terminal-protected VGGVG monomers
43 differently self-aggregate in water (Figures 1-2), to verify if these different morphologies slowly
44 evolve with time and to find the conditions for achieving the best PoAP-ELPs interaction, we have
45 performed a set of experiments, using three different aging times for both monomer types, (see
46 ‘Supplementary Materials’ comprehensive of S1-S5 Figures).
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51 Results lead to important outcomes on the use of Pt/PoAP/ELP for trimer detection.
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3 In particular, the unprotected pentapeptide adsorbs on PoAP mainly via H-bonds involving
4 carbonyls of either side, therefore, fresh adsorption leaves the prominent aliphatic component,
5 likely resulting by side chains exposed outward, preventing further adhesion. A suitable ageing time
6 was required for the monomer sequence to elongate, most likely via head to tail interaction, to a
7 point that a proper number of carbonyls could be left unbounded on PoAP surface and exposed
8 outwards for the subsequent detection of the trimer fibrils. The implicit, collateral, discovery was
9 that monomer sequences, too short to self-assemble into amyloids, can still aggregate into
10 unexpected supra-molecular features, with time and by external inducers as the external surface of
11 Pt/PoAP electrode, immersed in the monomer solutions (Figure S1).
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18 The terminal protected pentapeptide adsorbs on PoAP mainly via hydrophobic forces, involving
19 ELP' aliphatic side chains and PoAP' aromatic moieties, therefore, free carbonyls were mostly
20 available outward, without any need to age the monomer solution. Therefore, the influence of time
21 on self-aggregation was not further investigated. However, the 'progressive coverage' of PoAP
22 (decreasing Pt/N ratio) was found to correlate with the 'ageing' time of the monomer' solution
23 (Figure S3).
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29 The adsorption method has provided precious information on the polymer-peptide interaction, on
30 their ability of mutual recognition, either via H-bonds between interfacing carbonyls and
31 hydrophobic forces involving ELP' aliphatic side chains and PoAP' aromatic moieties. These forces
32 are exactly the same as those driving the self-assembly of hydrophobic peptides of the type
33 (XxxGlyGlyZzzGly) (Xxx,Zzz= Val, Leu), into extended β -sheet amyloids [30,39,40]. In
34 particular, carbonyls as ending groups and aromatic moieties of PoAP chains appear to modulate
35 the anchoring of H₂-VGGVG-OH (unprotected) and Boc-VGGVG-OEt (terminal protected)
36 peptides, respectively.
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43 Based on these premises, Figures 6 and 7, selected among the relevant supplementary figures, are
44 reported to represent the best Pt/PoAP/ELP combination of each monomer for sensing amyloid
45 fibrils.
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49 Figures 6 compare XPS C1s spectra and AFM images of the Pt/PoAP/ELP electrode (resulting from
50 Pt/PoAP exposition to 'unprotected' VGGVG solutions aged for 15 days), before (6a,b) and after
51 (6c,d) detection of (VGGVG)₃. Similarly, Figures 7 compare XPS C1s spectra and AFM images of
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Pt/PoAP/ELP electrodes (resulting from Pt/PoAP exposition to 'protected' VGGVG 'fresh' solutions), before (7a,b) and after (7c,d) detection of (VGGVG)₃.

Both figures testify a considerable attachment of the trimer fibrils on the surface of both selected Pt/PoAP/ELP systems, both having oxygenated functionalities properly exposed outward, as explained in supplementary materials and in points a, b of the above summary.

To verify if unwanted precipitation could be contributing to the attachments of ELP monomers on Pt/PoAP surfaces and be misinterpreted for specific adsorption, experiments with the 'protected' monomer at different ageing time were repeated using platinum foil as interacting surface. AFM images indicate no specific interactions between Pt and ELPs (Figure S6).

However, as for the experiments with fresh PoAP in Figures 3 and for the fresh VGGVG in S1, it would be expected a very tiny ELP adsorption also on platinum, not detectable by AFM. In fact, as revealed by surface mass spectrometry [38], the growth of PoAP chains, at the platinum sites during CV electro-synthesis, was reported to begin with the nitrogen atom of the oxidized monomer, oAP. Thus, a likely interaction with nitrogen, ELP-specific element, could be only indicative of Pt/PoAP synergy and of the benefit of using platinum substrate for ELP detection, vide infra.

Given these outcomes, in order to complete our study, in view of preventing also the possible occurrence of local detachments favored by the subsequent adsorbing steps of the procedure and make the stratified assembly easier to be prepared, more reproducible and possibly more efficient, the adsorption of ELP monomer on PoAP surface, as method of immobilization, was changed to entrapping at or near PoAP surface, during CV electrosynthesis.

It is worth noting that the template electro-polymerization of poly o-aminophenol was successfully attempted with specific proteins [55], therefore, we sought to adapt to our purposes the same procedure, normally adopted for the *surface imprinting*. Thus, ELP monomers, either terminal protected and unprotected, were added during CV electro-synthesis of the ultrathin PoAP membrane to be therein entrapped as surface receptors, as detailed in the next paragraph. The whole system was tested by XPS and AFM, before and after immersion in the aged suspension of trimer fibrils, also considering the influence of the underneath platinum.

d) Study of PoAP and (VGGVG)₃ interactions, mediated by VGGVG monomers entrapped on PoAP surfaces during CV electro-synthesis: influence of VGGVG terminal groups

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3 As anticipated, this procedure recalls the bi-dimensional MIP methodology [43,46,55] here revised
4 to entrap, irreversibly, each monomer without its removal at the end of the electro-synthesis. The
5 expectation was to have the monomer(s) firmly anchored at (or near at) the polymer surface, with
6 the functional groups of either side not fully engaged in reciprocal bonds and thus available to
7 intercept longer ELP sequences, as those of the trimer' type.
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11 Figures 8 show the CV electro-synthesis performed as usual, with the same number of scans, 1 to
12 20 cycles, used for insulating PoAP [25-27] with addition of unprotected (a) and protected (b)
13 VGGVG at the end of the first scan.
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17 In comparison to the CV growth of insulating PoAP [26], the CVs reported in Figure 8a,b show a
18 current re-increase after the addition of monomer(s) over the almost complete attenuation normally
19 recorded after the first cycle. The increase, of lower entity, for the protected monomer, may signify
20 're-exposure' of platinum sites, electro-active for oAP polymerization, due to 'displacement and /or
21 re-alignment' of polymer chains deposited during the first cycle.
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26 The AFM images of Figure 9a, very similar to those of insulating PoAP, suggest that unprotected
27 monomer is smoothly incorporated within the polymer chains without altering the PoAP
28 morphology. In parallel, carbon spectra show additional contribution for the monomer presence, but
29 with no appreciable changes of the overall carbon shape as well as of the Pt/N ratio, indicative of
30 substrate coverage. The surface offered to fibrils by the layered Pt/PoAP/ELP-unprotected shows an
31 increment of interacting groups, carbonyl- and carboxyl-like with respect to the 'inactive' Pt/PoAP
32 freshly prepared.
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38 On the other side, AFM images and XPS spectra of Figure 9b indicate a less compact and
39 homogeneous deposit when the protected monomer is added during PoAP electro-synthesis and
40 evident changes of the carbon and platinum peak shapes, respectively. In particular, Figure 9b
41 shows a different relative intensity of the carbon peaks compared to Figure 9a with abatement of
42 aliphatic components and further increment of carbonyl/carboxyl groups and a double chemical
43 state for platinum. Going more in detail, the curve-fitting results, shown in Table 2, indicate the
44 main carbon components of Figures 9a,b, fitted with the same peak parameters, to have similar
45 chemical state assignments, eventually including closely-spaced chemical states, but remarkably
46 different in their relative intensity. Moreover, the usual 4f doublet of metallic platinum [27] is
47 shown to be split in figure 9b, the component at the higher BE, unexpectedly, lying in the energy
48 interval characteristic of oxidized platinum. Taken together with the less marked increase of the
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3 anodic current of Figure 8b, these results may imply a direct interaction of the protected monomer
4 with the platinum substrate causing a partial steric hindrance towards oAP oxidation, most likely,
5 by means of the aliphatic protecting groups.
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9 A possible explanation from these preliminary results could be given considering the monomer(s)
10 influence on PoAP deposition, within the second and the twentieth cycle, as clearly perceived from
11 the CVs comparison of Figures 8. In fact, as known from previous studies, at the end of the first
12 cycle the platinum sites are almost, but not fully saturated yet, whereas oAP oxidation, followed by
13 attachment of short PoAP chains in vertical alignment to best accommodate vicinal chains, is found
14 to be completed at around the twentieth cycle, when the current is fully attenuated [25-27]. Thus,
15 ELP monomer(s) added at the end of the first cycle, upon its entrapping inside PoAP, could
16 interfere by displacing the deposited chains, by fastening their alignment and, eventually, by
17 interacting with the electro-active platinum sites, uncovered by this induced displacement/alignment
18 of PoAP chains.
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25 As for the adsorption method, the whole matter can be better reasoned by defining the interacting
26 surfaces seen by the fibrils, therefore, the performance of the two systems in the presence of
27 (VGGVG)₃ fibrils was investigated.
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31 Figures 10 compare XPS C1s regions and AFM images of the two Pt/PoAP/ELP systems made of
32 unprotected (10a) and protected (10b) VGGVG monomers, after interaction with the trimer
33 (VGGVG)₃ in form of fibrillar aggregates. Remarkably, Figures 10a,b undoubtedly prove the
34 ability of both systems to detect fibrillar aggregates. (VGGVG)₃ fibrils are easily recognizable in
35 AFM images and from XPS analysis, with both C1s profiles recalling that of the trimer, given the
36 massive fibrils adsorption. As for Figures 9, the carbon spectra of Figures 10 were resolved into
37 components by curve-fitting. Results confirm the significant presence of ELP components for both
38 cases, although a greater interactions was observed for the system made of protected monomer.
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45 Although further investigation are required to fully understand the mechanisms governing these
46 Pt/PoAP/ELP interactions, nevertheless layered systems based on monomers entrapments prove to
47 be the most promising as bio-detectors, performing with high specificity towards amyloid fibrils.
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51 The greater degree of fibrils interaction with the system made of protected monomer can be
52 explained by its wider bio-receptors distribution along the exposing surface due to different
53 'entrapping' sites of the monomer itself, including platinum substrate, acting in synergy. It is
54 equally important to preserve the intact coverage of the electrode substrate, better ensured by the
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3 unprotected monomer, for avoiding its poisoning. Moreover, for a good fibrils detection, a too
4 massive absorption is not so recommended if we consider that a possible detachment could take
5 place subsequently, perhaps even during ongoing measurements.
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9 10 **CONCLUSIONS**

11 All together results from the present study provide unequivocally evidence of the forces that are
12 driving the mutual interaction of the investigated biomolecules.
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15 The insulating PoAP, electro-synthesized on platinum has proven useful to efficiently adsorb/entrap
16 ELP monomers on its surface and can be used as receptors of ELP fibrils, relying on interacting
17 forces, similar to those inducing ELPs self-assembly, favored by the anchoring of the monomer
18 sequences.
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22 The best Pt/PoAP/ELP assembly to detect ELP fibrils, (VGGVG)₃, requires the outer surface
23 exposing the right number and disposition of 'anchoring' carbonyls, as also demonstrated by the
24 augmented efficacy of Pt/PoAP electrode, alone, when stored in air for days, due to the increment
25 of carbonyls by post-synthesis oxidation of -C=N< groups.
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29 In the perspective of proposing an optimal performing biosensor for amyloid detection, various
30 combinations of Pt/PoAP/ELP assembly were investigated, evaluating the influence of the
31 following variables:
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- 35 • aging time of the VGGVG monomers solution influences aggregation mechanisms of
36 (VGGVG)_n sequences, even for the shortest ones (n=1), thus providing new information on
37 the kinetics of aggregation and on the mechanism of fibrils' nucleation
38
- 39 • presence of protective terminal groups induces a different kind of interaction with the PoAP
40 substrate: hydrophobic forces were prominent for Boc-VGGVG-OEt, whereas H-bonds
41 between carbonyls were prevalent for H₂-VGGVG-OH
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- 45 • two methods of immobilization of ELP monomers, namely the adsorption method and the
46 surface imprinting method based on bi-dimensional MIP, were compared by exposing
47 Pt/PoAP/ELP electrodes to VGGVG trimer in order to evaluate their efficacy in recognizing
48 amyloid fibrils in solution .
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3 Results show the advantage of the modified bi-dimensional MIP methodology, fast to prepare and
4 to reproduce, to efficiently retain on Pt/PoAP surface the imprinted ELP monomer(s) as bio-
5 detectors. Indeed, their ability to detect fibrillar aggregates was amply proved, in this work.
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8 We therefore believe that, if properly conveyed, these MIP-based systems have the right
9 characteristics to be the receptor components of a biosensor device for neurodegenerative diseases,
10 as Alzheimer Disease.
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22

23
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26 during the PhD thesis of M.E.E Carbone (A.Y. 2015-2016).
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31 **CONFLICT OF INTEREST**

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34 The authors declare that there are no known conflicts of interest regarding the publication of this
35 article.
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For Peer Review

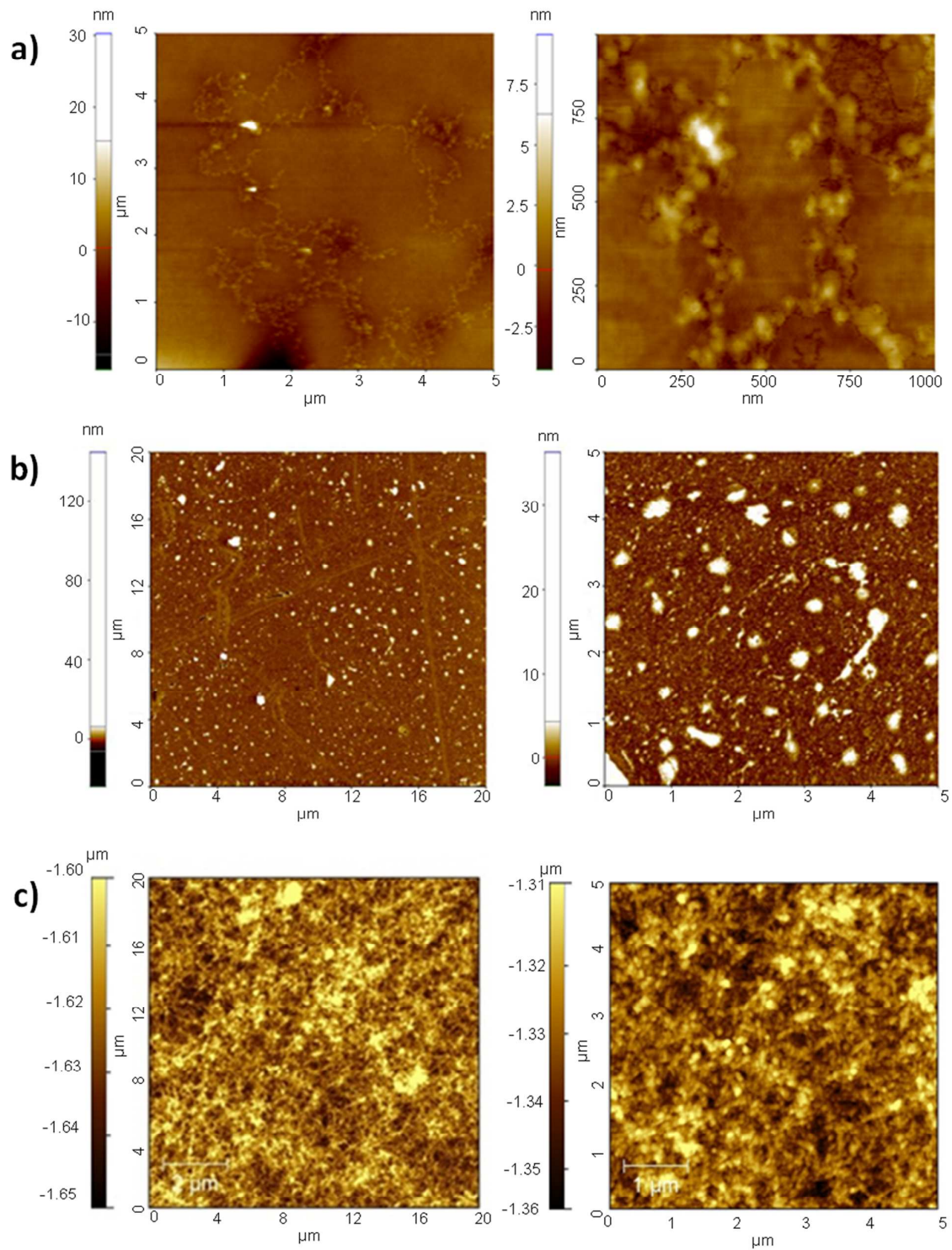


Fig.1 AFM images of VGGVG (a) BocVGGVGOEt (b) and (VGGVG)₃ (c) water suspension (0.1 mg/mL) deposited on silicon wafer.

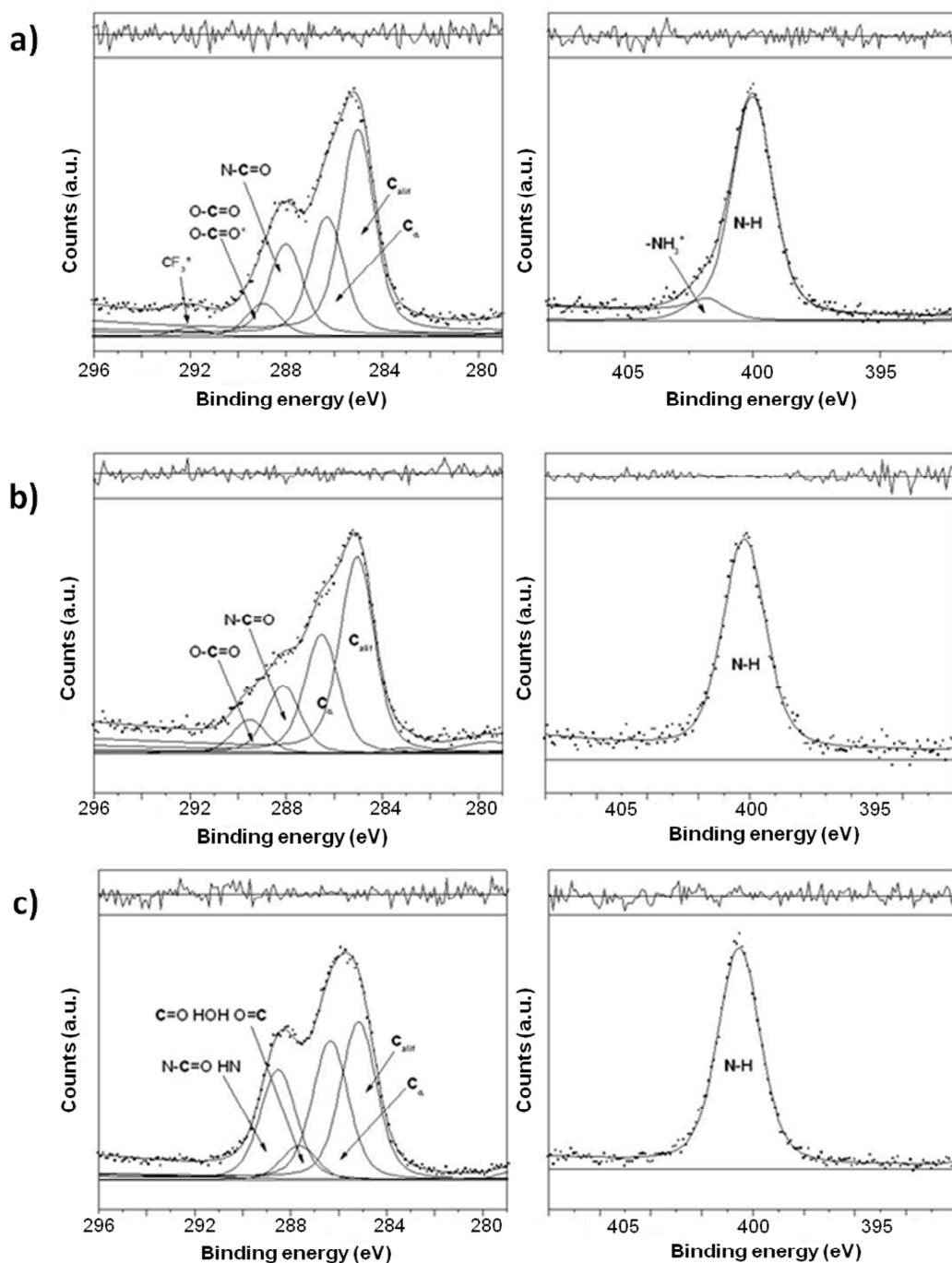


Fig.2 XPS curve-fitted C1s and N1s spectra of VGGVG (a) BocVGGVGOEt (b) and (VGGVG)₃ (c) water suspension (0.1 mg/mL) deposited on silicon wafer. Curve-fitting results are reported in Table 1 The O1s regions of monomer deposits (2a,2b) are not reported due to the substrate signals interference. Results of the curve-fitted O1s region of the trimer deposits(2c) are reported in Table 1

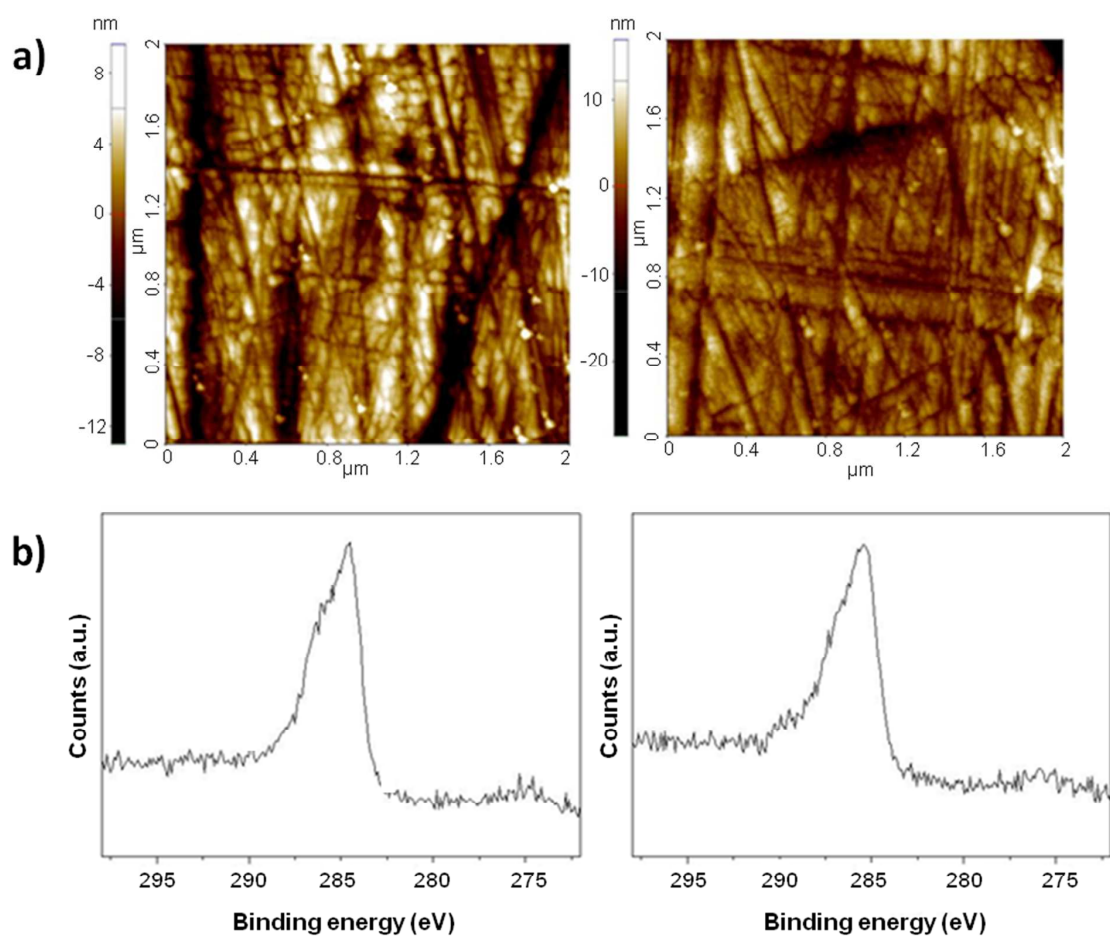


Fig.3 Interaction of insulating PoAP with (VGGVG)₃ solution: AFM images and XPS C1s spectra of the PoAP before (on the left) and after (on the right) exposure to (VGGVG)₃ solution.

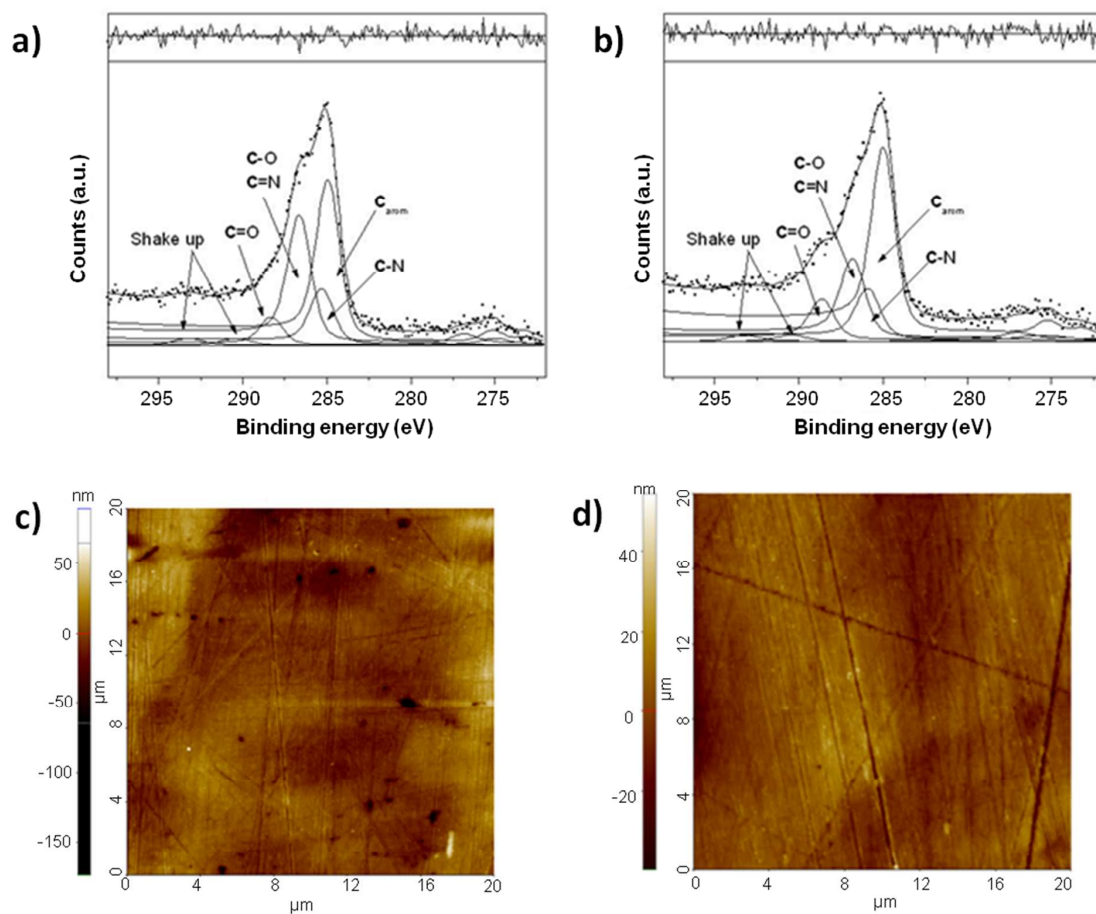


Fig.5 XPS curve-fitted C1s region of the insulating PoAP analyzed immediately after the electro-synthesis (a) and after 15 days of aging in air at room temperature (b) and relevant AFM images (c,d). Curve-fitting results are reported in Table 2.

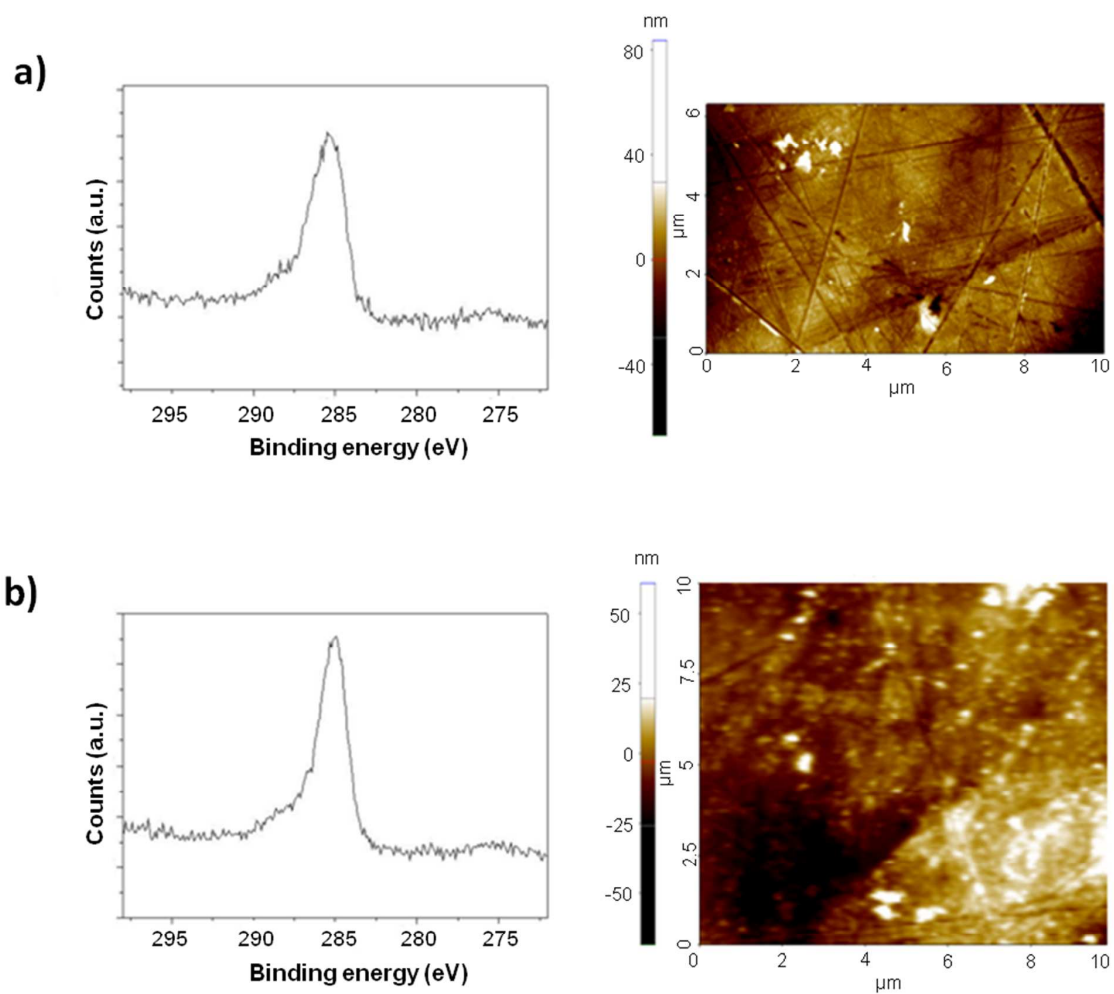


Fig.6 XPS C1s spectra and AFM images of the Pt/PoAP electrodes exposed to 15 days aged VGGVG solutions (on the left); XPS C1s spectra and AFM images of the detection of (VGGVG)₃ (on the right).

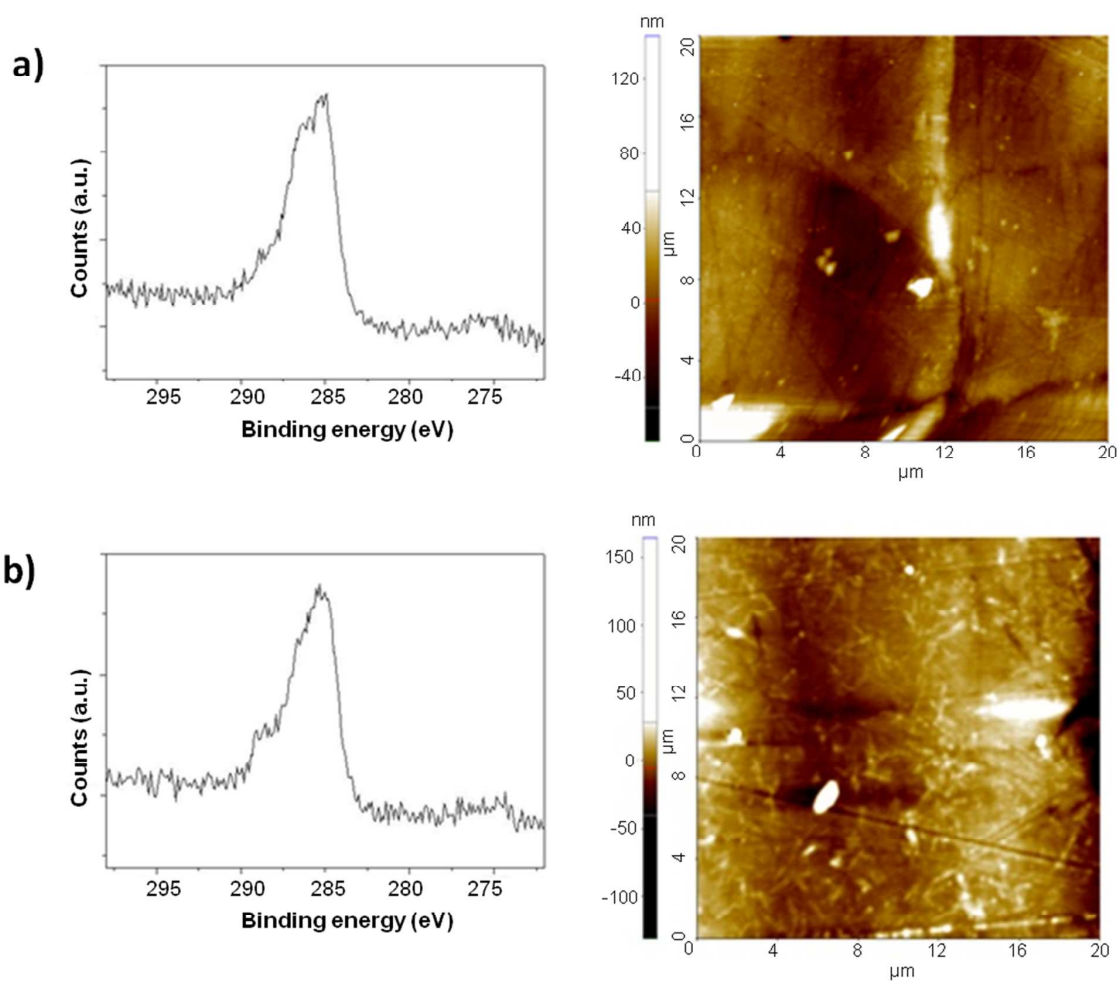


Fig.7 Interaction of insulating PoAP with BocVGGVGOEt solutions: XPS C1s spectra and AFM images of the Pt/PoAP electrodes exposed to fresh protected-VGGVG solutions (on the left); XPS C1s spectra (on the left) and AFM images (on the right) of the detection of (VGGVG)₃ (on the right).

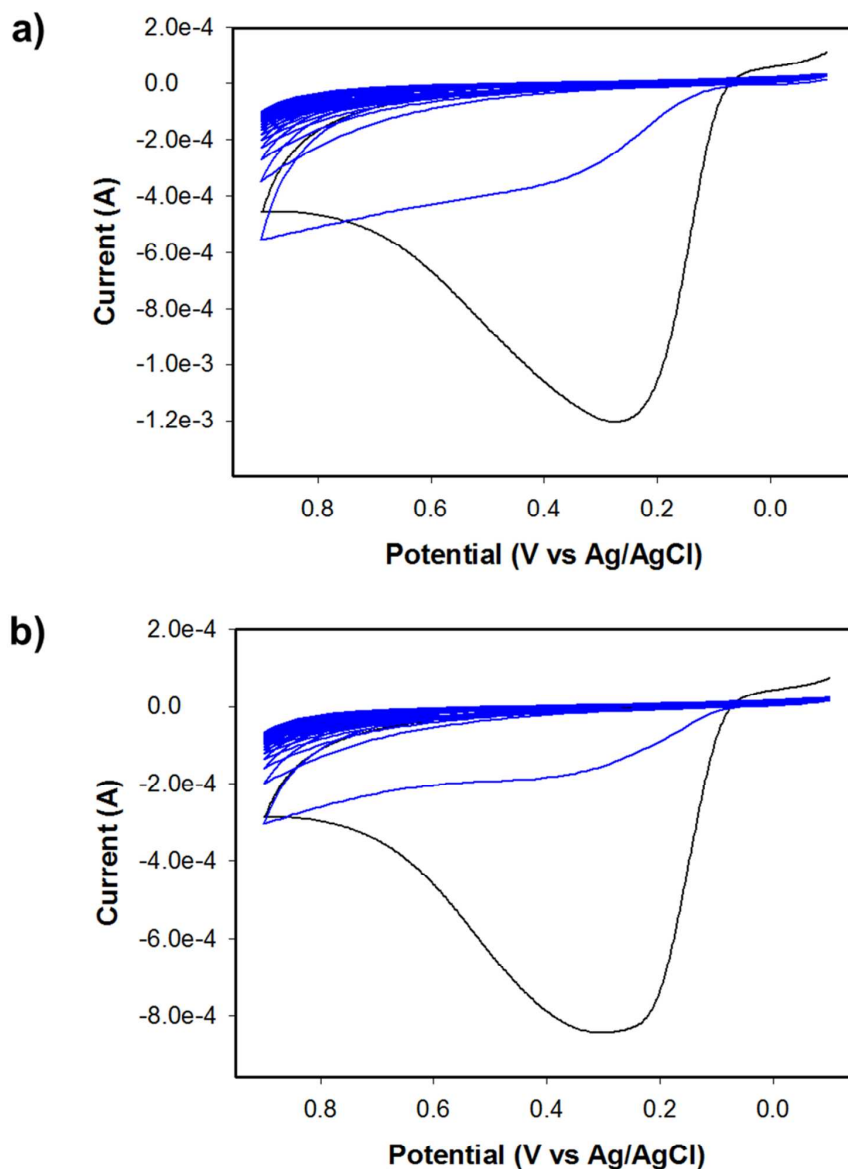


Fig.8 Cyclic voltammetry of electrosynthesis of insulating PoAP on platinum electrode with a scan rate of 50 mV/s: the first voltammetric cycle (in Black) is registered in 5 mM oAP solution in phosphate buffer ($I = 0.1M$, $pH = 7.0$), while the other nineteenth scan are acquired after the addition of an aqueous solution of VGGVG (A) and BocVGGVGOEt (B) at a concentration of 0.1 mg/mL.

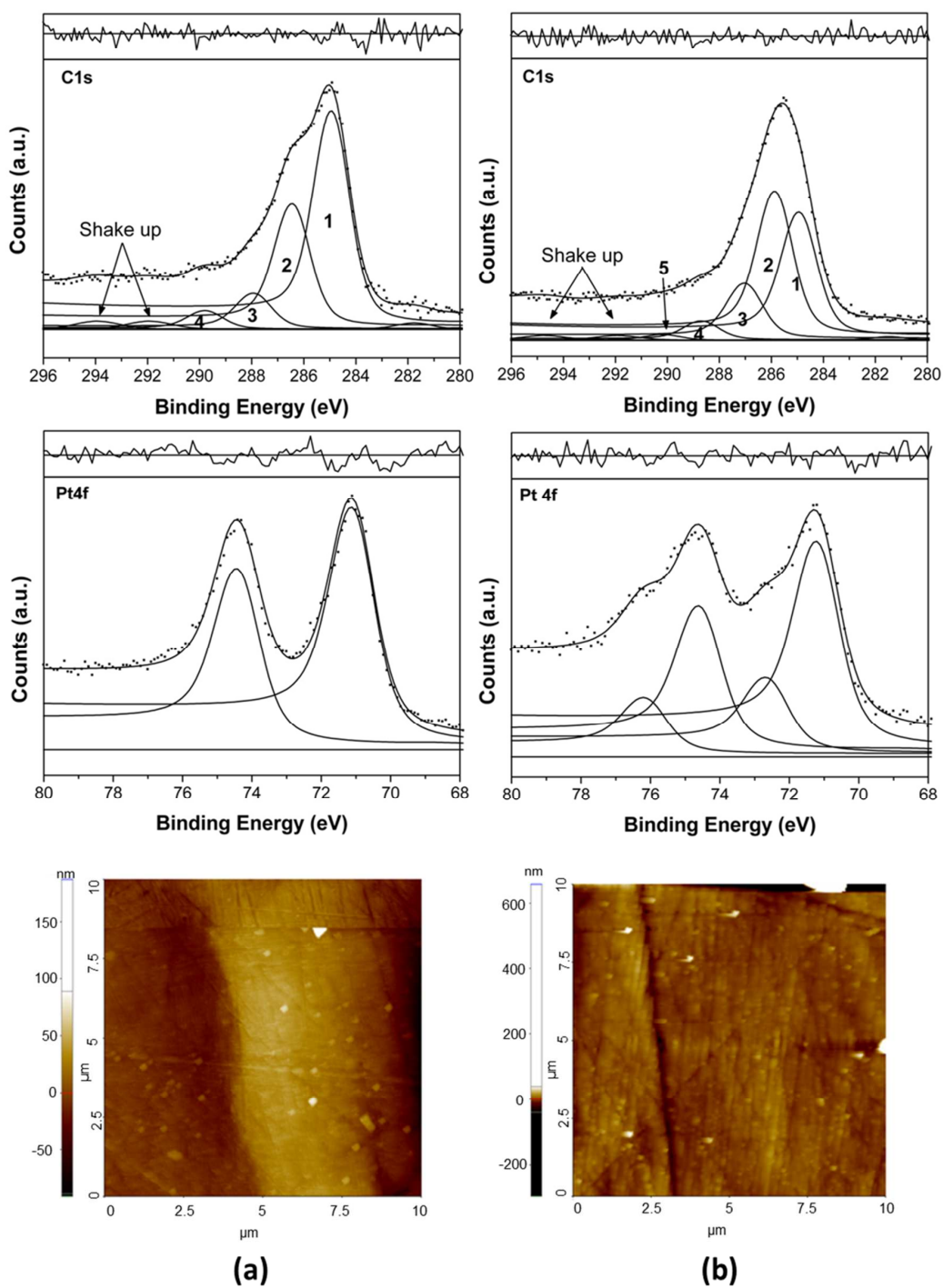


Fig.9 XPS curve fitted C1s and Pt4f regions of PoAP with entrapped VGGVG (A) and BoCVGGVGOEt (B) and relevant AFM images at 10x10 μm magnification. Curve-fitting results are reported in Table 2.

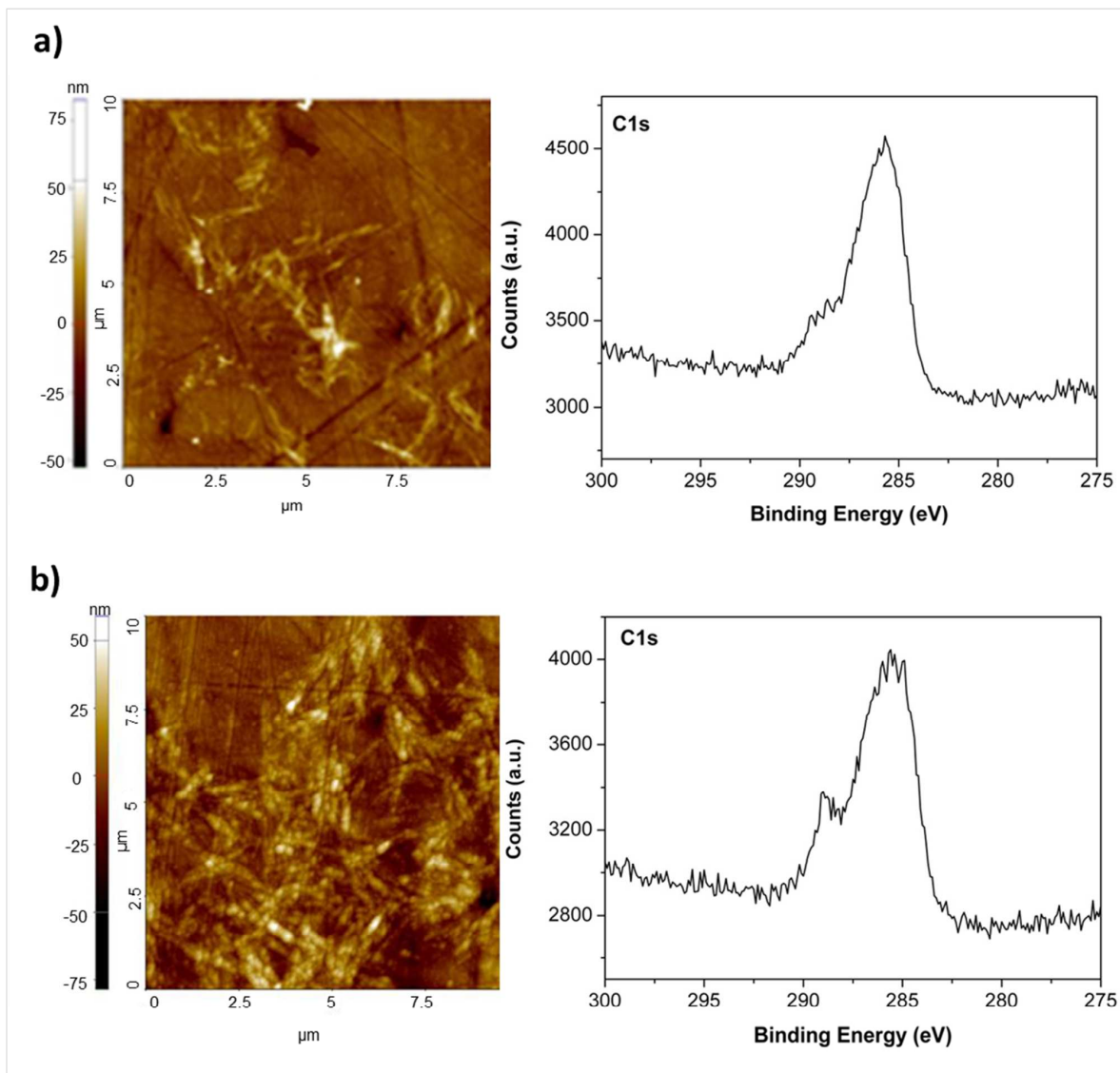


Fig.10 AFM images and XPS C1s signals of Pt/PoAP/VGGVG (A) and Pt/PoAP/BoCVGGVGOEt (B) and after the detection of (VGGVG)₃ fibrils.

Table 1. Curve fitting results of reference XPS spectra reported in Figures 2

Sample	Element	Peak number	BE corr. (eV)	FWHM (eV)	Corrected area (arbitrary unit)	Number of atoms for repeat unit	Assignment
$\underline{\text{VGGVG}}/\text{Si}$	C1s	1	285.0	1.66	1763.8	6.0	C (CH)
		2	286.3	1.66	1474.2	5.0	C _α
		3	288.0	1.66	1149.9	3.9	N-C=O
		4	289.0	1.66	410.9	1.4	O-C=O O-C=O*
		5	292.0	1.66	115.1	0.4	CF ₃ *
	N1s	1	400.0	1.90	1337.5	4.5	N-H
		2	401.9	1.90	132.4	0.5	-NH ₃ ⁺
$\underline{\text{BOCVGGVGOEt}}/\text{Si}$	C1s	1	285.0	1.69	2428.8	10.0	C (CH)
		2	286.5	1.69	1696.9	6.9	C _α
		3	288.1	1.69	961.2	3.9	N-C=O
		4	289.5	1.69	480.6	2.0	O-C=O
	N1s	1	400.2	1.98	1214.5	5.0	N-H
$\underline{\text{(VGGVG)}_2/\text{Si}}$	C1s	1	285.0	1.69	1439.10	6.0	C (CH)
		2	286.2	1.69	1202.12	5.0	C _α
		3	287.5	1.69	300.68	1.2	C=O...HOH...O=C
		4	288.4	1.69	936.08	3.9	N-C=O...HN
	O1s	1	531.6	1.64	306.49	1.2	C=O...HOH...O=C
		2	532.3	1.64	946.84	3.9	N-C=O...HN
		3	533.2	1.64	269.37	-	H ₂ O
	N1s	1	400.4	1.89	1202.20	5.0	HN-C=O

Table 2. Curve fitting results of XPS spectra reported in Figures 4, 5 and 9

Sample	Element	Peak number	BE corr. (eV)	FWHM (eV)	Corrected area (arbitrary unit)	At%	Assignment
<u>VGGV/G_{1/2}/aged PoAP</u>	C1s	1	284.8	1.55	7471.56	50.7	C (CH)
		2	285.2	1.55	1288.20	8.7	C _α
		3	286.0	1.55	2768.40	18.8	C-O C=N C-N ⁺
		4	287.2	1.55	1343.04	9.1	C=O
		5	288.7	1.55	1136.64	7.7	COOH
		6	290.3	2.00	373.58	2.5	Shake up
		7	293.1	2.00	373.58	2.5	Shake up
<u>PoAP</u>	C1s	1	284.8	1.55	5435.88	41.4	C arom
		2	285.2	1.55	1864.10	14.2	C-N
		3	286.5	1.55	4256.68	32.4	C-O C=N
		4	288.2	1.55	924.47	7.0	C=O
		5	290.3	2.00	318.92	2.5	Shake up
		6	293.1	2.00	318.92	2.5	Shake up
<u>Aged PoAP</u>	C1s	1	284.8	1.55	4077.56	40.6	C arom
		2	285.2	1.55	1530.68	15.2	C-N
		3	286.5	1.55	2928.41	29.2	C-O C=N
		4	288.4	1.55	1060.87	10.6	C=O
		5	290.3	2.00	218.95	2.2	Shake up
		6	293.1	2.00	218.95	2.2	Shake up
<u>Pt/PoAP/VGGVG</u>	C1s	1	284.8	1.55	8339.24	52.0	C (CH)
		2	286.3	1.55	4773.92	29.7	C-O + C-N + C _α
		3	287.8	1.55	1380.03	8.6	N-C=O C=O + C=N
		4	289.6	1.55	719.88	4.5	C=O...H ₂ O
		5	291.8	2.00	416.96	2.6	Shake up
		6	293.8	2.00	416.96	2.6	Shake up
	Pt4f	1	71.3	1.45	514.54	57.1	Pt4f _{7/2}
		2	74.6	1.45	385.90	42.9	Pt4f _{5/2}
<u>Pt/PoAP/BoCVGGVGOEt</u>	C1s	1	284.8	1.55	5752.24	34.4	C (CH)
		2	285.7	1.55	6693.12	40.0	C-N + C _α
		3	286.9	1.55	2567.25	15.4	C-O + C=O + C=N
		4	288.6	1.55	868.96	5.2	N-C=O
		5	290.0	1.55	265.22	1.6	C=O...H ₂ O
		6	291.8	2.00	287.61	1.7	Shake up
		7	294.6	2.00	287.61	1.7	Shake up
	Pt4f	1	71.1	1.45	270.80	43.2	Pt4f _{7/2}
		2	72.5	1.45	95.46	15.2	PtOx 4f _{7/2}
		3	74.5	1.45	189.68	30.2	Pt4f _{5/2}
		4	76.0	1.45	71.59	11.4	PtOx 4f _{5/2}

FIGURE CAPTIONS

Fig.1 AFM images of VGGVG (a) BocVGGVGOEt (b) and (VGGVG)₃ (c) water suspension (0.1 mg/mL) deposited on silicon wafer.

Fig.2 XPS curve-fitted C1s and N1s spectra of VGGVG (a) BocVGGVGOEt (b) and (VGGVG)₃ (c) water suspension (0.1 mg/mL) deposited on silicon wafer. Curve-fitting results are reported in Table 1. The O1s regions of monomer deposits (2a,2b) are not reported due to the substrate signals interference. Results of the curve-fitted O1s region of the trimer deposits(2c) are reported in Table 1.

Fig.3 Interaction of insulating PoAP with (VGGVG)₃ solution: AFM images and XPS C1s spectra of the PoAP before (on the left) and after (on the right) exposure to (VGGVG)₃ solution.

Fig.4 XPS curve-fitted C1s (a), O1s (b), N1s regions (c) and AFM images 5x5 μm, (d) of insulating PoAP aged 15 days and then exposed for 6 h at 37 °C to the solution of (VGGVG)₃. Curve-fitting results are reported in Table 2.

Fig.5 XPS curve-fitted C1s region of the insulating PoAP analyzed immediately after the electrosynthesis (a) and after 15 days of aging in air at room temperature (b) and relevant AFM images (c,d). Curve-fitting results are reported in Table 2.

Fig.6 XPS C1s spectra and AFM images of the Pt/PoAP electrodes exposed to 15 days aged VGGVG solutions (on the left); XPS C1s spectra and AFM images of the detection of (VGGVG)₃ (on the right).

Fig.7 Interaction of insulating PoAP with BocVGGVGOEt solutions: XPS C1s spectra and AFM images of the Pt/PoAP electrodes exposed to fresh protected-VGGVG solutions (on the left); XPS C1s spectra (on the left) and AFM images (on the right) of the detection of (VGGVG)₃ (on the right).

Fig.8 Cyclic voltammetry of electrosynthesis of insulating PoAP on platinum electrode with a scan rate of 50 mV/s: the first voltammetric cycle (in Black) is registered in 5 mM oAP solution in phosphate buffer (I = 0.1M, pH = 7.0), while the other nineteenth scan are acquired after the

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3 addition of an aqueous solution of VGGVG (A) and BocVGGVGOEt (B) at a concentration of 0.1
4 mg/mL.
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7 **Fig.9** XPS curve fitted C1s and Pt4f regions of PoAP with entrapped VGGVG (A) and
8 BoCVGGVGOEt (B) and relevant AFM images at 10x10 μm magnification. Curve-fitting results
9 are reported in Table 2.
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13 **Fig.10** AFM images and XPS C1s signals of Pt/PoAP/VGGVG (A) and Pt/PoAP/BoCVGGVGOEt
14 (B) and after the detection of (VGGVG)₃ fibrils.
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17 **Table 1.** Curve fitting results of reference XPS spectra reported in Figures 2.
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19 **Table 2.** Curve fitting results of XPS spectra reported in Figures 4, 5 and 9.
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Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Interactions between elastin-like peptides (ELPs) and insulating poly ortho-aminophenol (PoAP) membrane investigated by AFM and XPS

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S1 Interaction of insulating PoAP with VGGVG solutions: XPS C1s spectra (on the left) and AFM images (on the right) of the Pt/PoAP electrodes exposed to fresh (a), 15 days (b) and 76 days (c) aged VGGVG solutions.

S2 Optical microscope image of the Pt/PoAP foils exposed to solutions of VGGVG 76 days aged (a) and of species present in aged (76 days) VGGVG solution deposited onto silicon substrate (b).

S3 XPS C1s spectra (on the left) and AFM images (on the right) of the detection of (VGGVG)₃ employing the Pt/PoAP/VGGVG electrodes obtained with fresh (a), 15 days (b) and 76 days (c) aged monomer solutions.

S4 Interaction of insulating PoAP with BocVGGVGOEt solutions: XPS C1s spectra (on the left) and AFM images (on the right) of the Pt/PoAP electrodes exposed to fresh (a), 15 days (b) and 76 days (c) aged VGGVG solutions.

S5 XPS C1s spectra (on the left) and AFM images (on the right) of the detection of (VGGVG)₃ employing the Pt/PoAP/BocVGGVGOEt electrodes obtained with fresh (a), 15 days (b) and 76 days (c) aged monomer solutions.

S6 AFM images of Pt in its pristine state (a) and after interaction with BocVGGVGOEt fresh (b); 15 days (c) and 76 days (d) aged solutions. **The sequence of experiments is to be compared with its analogue in S4.**

1
2
3 *Study of PoAP and (VGGVG)₃ interactions, mediated by VGGVG monomers pre-adsorbed on*
4 *PoAP surfaces from water solutions, differently aged, at ambient conditions: influence of VGGVG*
5 *terminal groups*
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7 *H₂-VGGVG-OH*
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9 Figures S1 shows the XPS spectra, limited to C1s regions, as the best resolved by curve-fitting of
10 Pt/PoAP surfaces after immersion into VGGVG solutions: a) fresh; b) aged for 15 days; c) aged for
11 76 days together with the relevant AFM images. By comparing each set with the reference XPS
12 spectra and AFM images of the 'as prepared' electrode, the progressive adsorption of the
13 unprotected monomer on Pt/PoAP by increasing its aging time in water, is confirmed by all
14 evidence, including the attenuation of the underlying platinum substrate, estimated by the parallel
15 decrease of Pt/N ratios, going from 0.84 (fresh) to 0.65 (15days) and 0.44 (76days), see Figures.
16

17 Moreover, focusing on details, interesting information are added on the ELP adsorption on PoAP
18 and on its self-aggregation mechanisms. In particular:
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- 20 • the very limited adsorption of the fresh monomer on Pt/PoAP surface recall the situation
21 previously encountered with the trimer suspension, see Figure 4, where the morphology of
22 the adsorbed layers was not really discernable by AFM;
23
- 24 • the change of C1s peak shape (Fig. S1a), even more remarkable in this case, better suggests
25 that PoAP-ELP interactions have occurred through C=O...H-peptide bonds, as revealed by
26 the carbonyls shifting towards higher BEs (i.e. appearance of carboxylic-like features
27 labeled in Figure 5b) and by the prominent aliphatic component, likely resulting by side
28 chains exposed outward, enhanced by XPS, preventing further adhesion;
29
- 30 • going ahead with ageing time, ELP adsorption progresses and the carbon 1s shape of
31 Pt/PoAP surfaces continues to change accordingly. The carbonyl and carboxyl intensities,
32 ratioed to aliphatic intensity increase with aged ELP, reaching a maximum in 15 days (Fig.
33 S1b). Up to there, the adsorbates, still unstructured but widely distributed, are better
34 detected by AFM, together with light patches here and there over the surface. The
35 augmented ELP coverage (Pt/N=0.65) can be considered as an indication of its aggregation
36 tendency, preannounced as head-tail alignment (Figure 1) of the unprotected sequence, to be
37 evolving over time while ageing in water, presumably, leading to 'longer chain' adsorption
38 on PoAP carrying additional carbonyls, available for further adsorption;
39
- 40 • Finally, the situation after 76 days is very impressive. During the long aging time, the
41 monomer self-aggregation has progressed to a point of producing the amazing supra-
42 molecular structure shown in Figure S1c. However, by comparing the optical images of
43 **Figure S2a** with the optical image of deposits on silicon wafer from the same solution
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(**Figure S2b**), it becomes immediately clear that it is the adsorption on Pt/PoAP to specifically promote the assembly of long-aged VGGVG into very long, interconnected, tubular fibres with dimensions of the order of microns, probably facilitated in their propagation by the anchoring mainly offered by carbonyl functionalities over the PoAP surface. The correspondent XPS analysis, shows a higher degree of coverage ($Pt/N = 0,45$) and a broad carbon 1s spectra regressed to a shape dominated by aliphatic components, indicative of a nearly saturated coverage, hindering further adsorption.

It was a matter then to see how the three modified electrodes would perform in the presence of the trimer fibrils suspended in water.

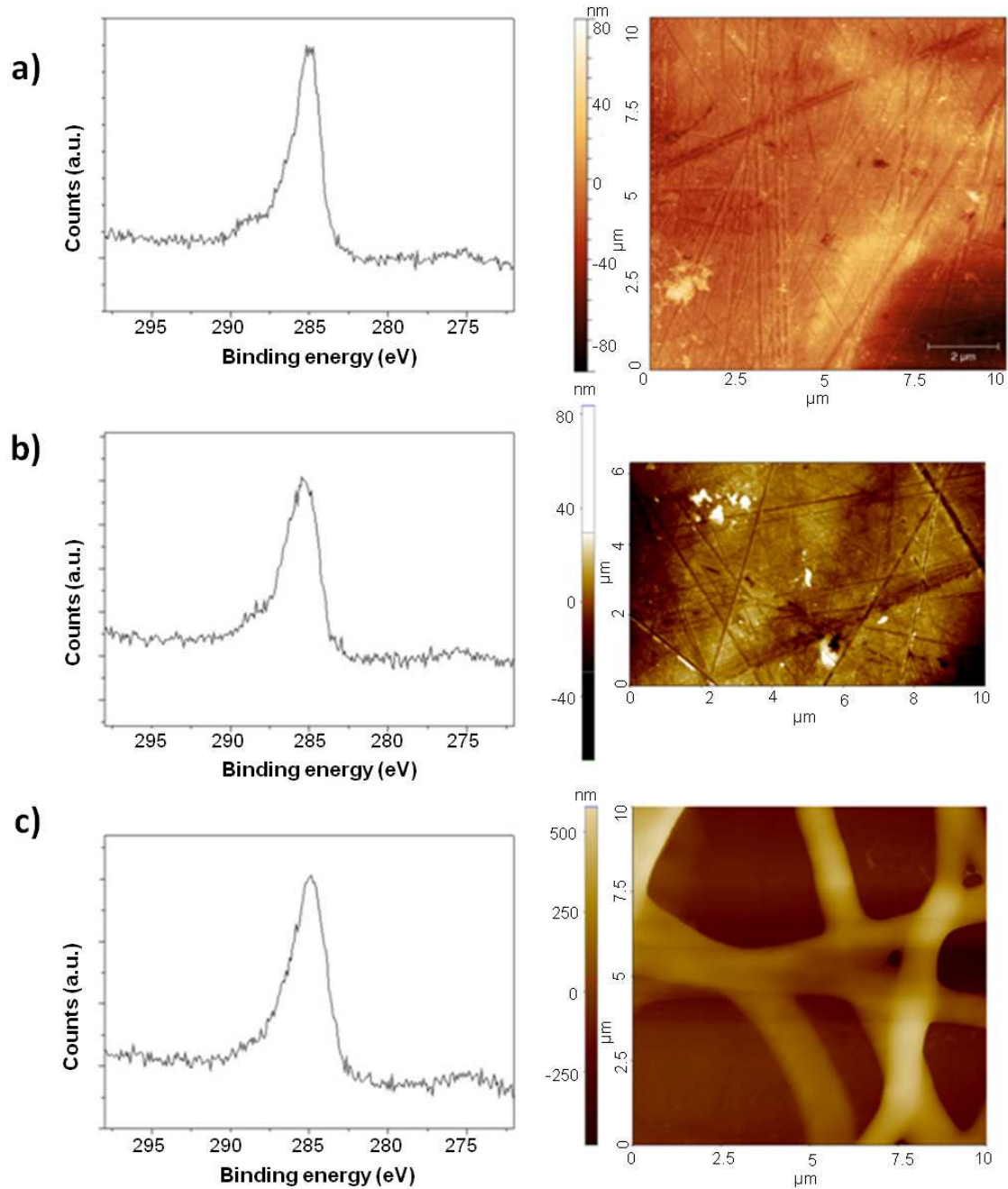
Figures S3 shows the carbon 1s sequences of the three Pt/PoAP/ELP surfaces, after interaction with $(VGGVG)_3$ suspended in water, and the corresponding AFM images. The figures are self-explanatory.

Figure S3a proves, similarly to the precedent 'as prepared' Pt/PoAP electrode of Figure 3, the trimer adsorption on the modified PoAP surface of figure S1a, mainly by XPS. The change of C1s shape testifies that adsorption has taken place to an extent that leaves unaltered the substrate morphology and the Pt/N ratio is roughly the same as before immersion into the trimer suspension.

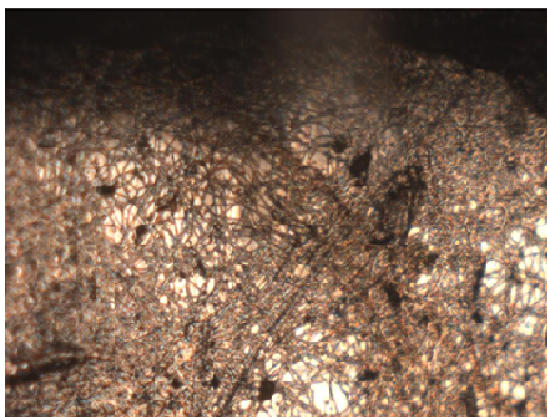
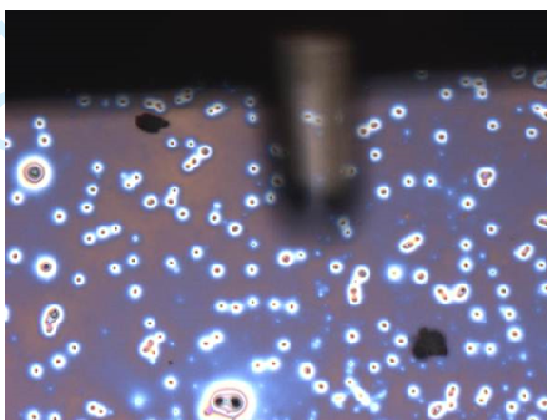
Figure S3b shows a massive attachment of aggregates also recognizable in previous deposits from trimer suspension imaged on silicon. This time, the trimer adsorption masks the underneath features, in parallel, XPS gives the ratio Pt/N reduced to 0.47, index of greater coverage in comparison to the reference surface of figure S1b.

In Figure S3c the magnified AFM image shows a kind of deterioration of the, previously intact, interconnected fibres of figure S1c while XPS shows a further narrowing of C1s peak around the aliphatic component. Taken with cautions, given the massive envelope of fibers, results from curve-fitting indicate the same Pt/N ratio as before, as if no trimer attachment has taken place. In reality, it could be that further interactions with the suspended trimer, inducing the deterioration of fibres, also leads to a concomitant detachment of some, less bounded, parts of their extended envelope.

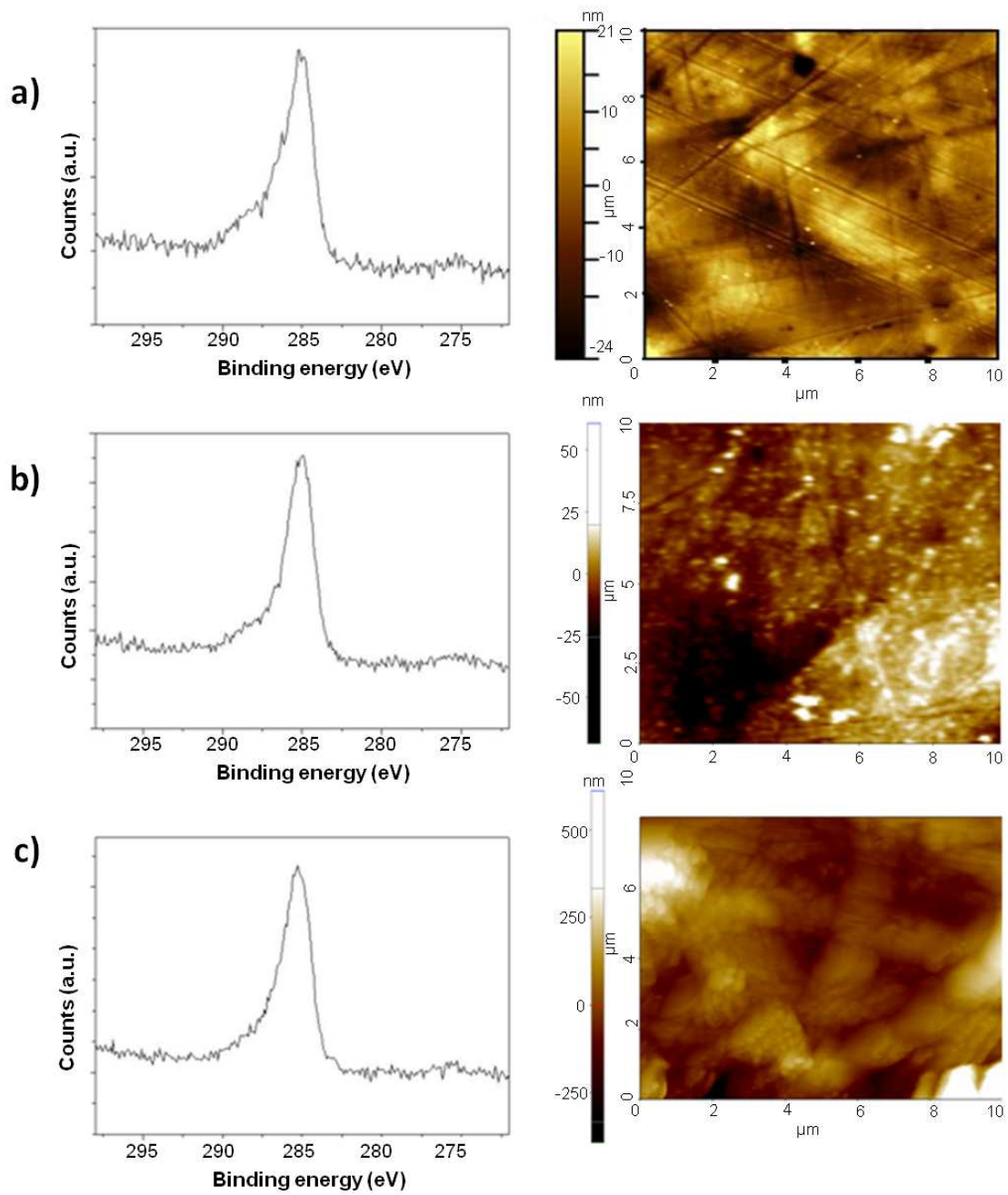
In order to complete this study and find the best Pt/PoAP/ELP combination for sensing amyloid fibrils, the same set of experiments was repeated with the same ELP sequence protected with terminal groups, as reported in the following paragraph.



S1 Interaction of insulating PoAP with VGGVG solutions: XPS C1s spectra (on the left) and AFM images (on the right) of the Pt/PoAP electrodes exposed to fresh (a), 15 days (b) and 76 days (c) aged VGGVG solutions.

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a)**b)**

S2 Optical microscope image of the Pt/PoAP foils exposed to solutions of VGGVG 76 days aged (a) and of species present in aged (76 days) VGGVG solution deposited onto silicon substrate (b).



S3 XPS C1s spectra (on the left) and AFM images (on the right) of the detection of (VGGVG)₃ employing the Pt/PoAP/VGGVG electrodes obtained with fresh (a), 15 days (b) and 76 days (c) aged monomer solutions.

BoC-VGGVG-OEt

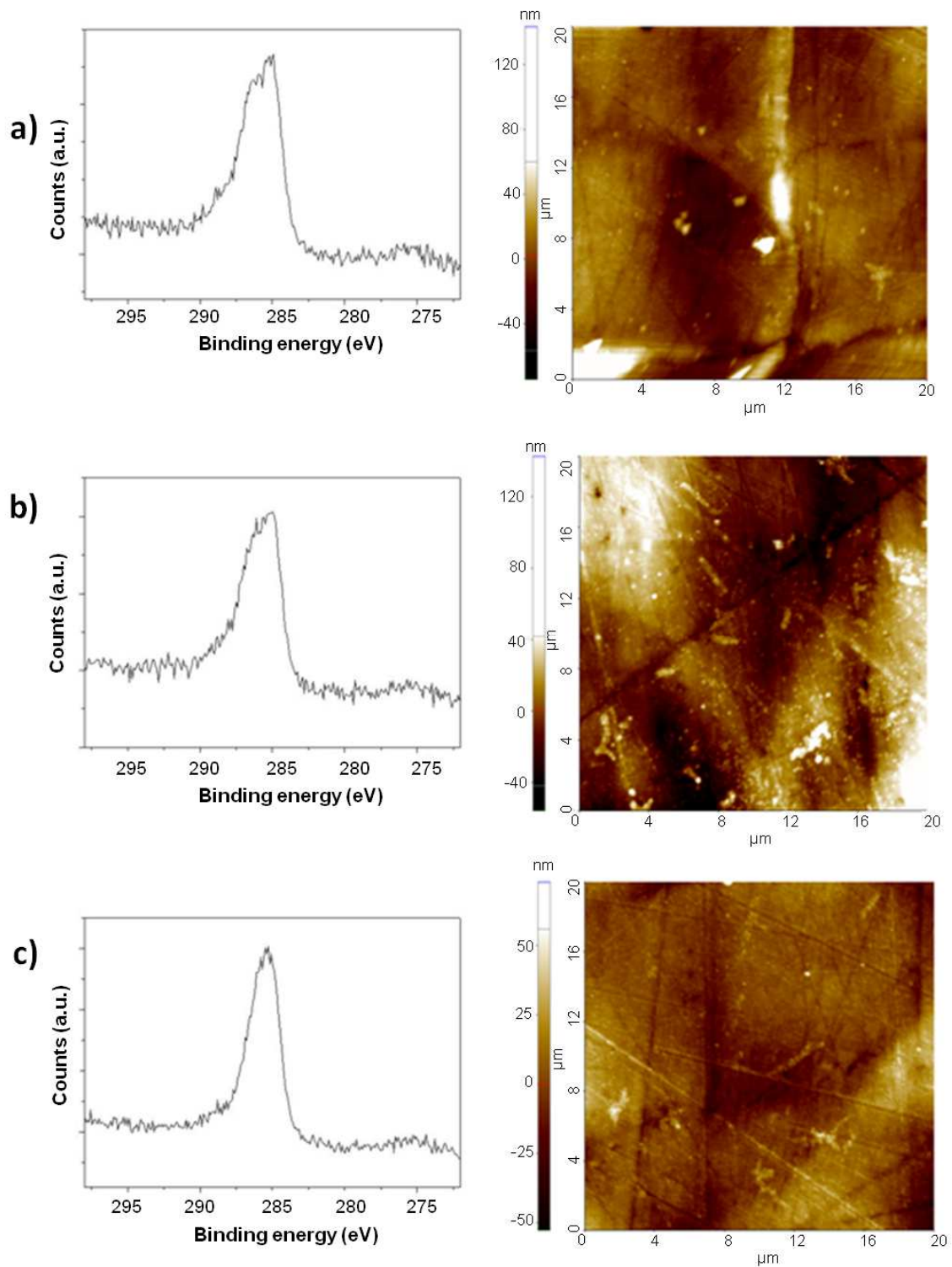
Figures **S4** and **S5** are resuming the same experiments with BoC-VGGVG-OEt, using the same experimental conditions, carried out for the unprotected pentapeptide.

Briefly, the two set of figures compare, by XPS and AFM, the Pt/PoAP surface modified by BoC-VGGVG-OEt adsorption from fresh (a), aged for 15 days (b) and long aged for 76 days (c) solutions, before (Figure 10) and after (Figure 11) interaction with the aged trimer suspension.

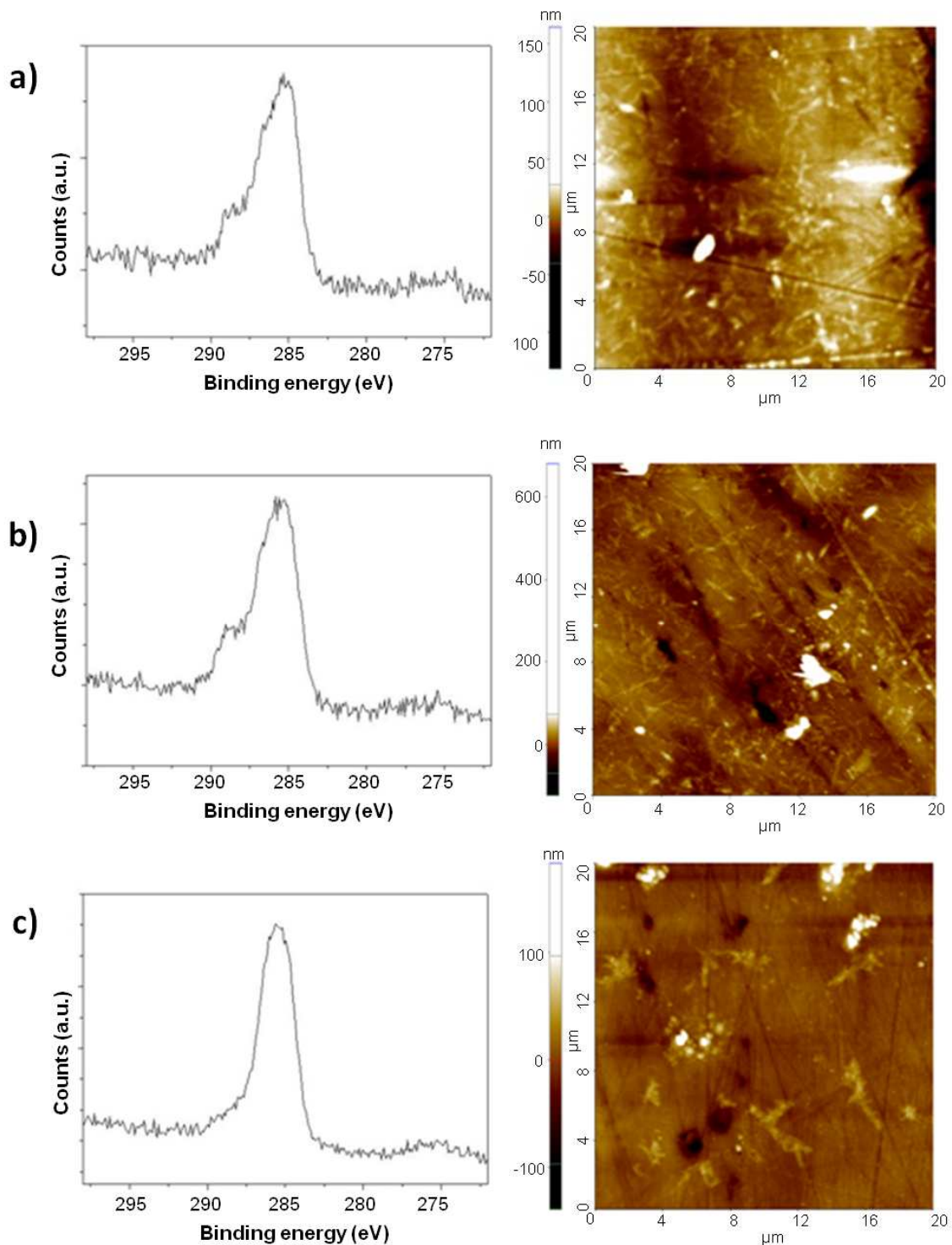
Going through the series, some similarities with the unprotected monomer are seen and regard the effect of ageing time, deducible also for the protected monomer by the correlation between the coverage degree of PoAP surfaces and changes of the C1s peak shape, either before and after trimer adsorption. However, the differences emerged are more important to deepen the understanding of the 'interaction' mechanisms under study, as hereafter discussed.

The first important 'difference' regards the PoAP-monomer interactions, in this case, mainly involving the aromatic repeat and the encumbering aliphatic terminal groups, respectively. In fact, limiting the analysis to the fresh and short-aged BoC-VGGVG-OEt solution, (leaving out long aged conditioning, as promoter of massive adsorption leading to coverage saturation even prone to detachment), it is easily noticed that C1s peak shapes of Figures S4a,b differ from the correspondent in Figures S1a,b of the unprotected monomer. In this case, particularly for the fresh solution, the oxygenated functionalities are those exposed outwards, thus oppositely to the its homologue, the protected monomer interacts with PoAP by means of hydrophobic forces.

Another important and consequent difference is shown along the series of Figures S5 where signs of the trimer adsorption are clearly identified already for the Pt/PoAP surface modified with BoC-VGGVG-OEt from fresh solution (Fig. S5a). Indeed AFM and XPS respectively show aggregate features and C1s peak shape resembling those reported in Figure 3, related to trimer deposits on silicon wafer from the same water suspension. Thus, pre-adsorption of the protected monomer on PoAP, both freshly prepared without any need of further ageing, occurs via hydrophobic forces, while the remaining groups prone to H-bonds make the outer surface very sensitive towards trimer fibrils.

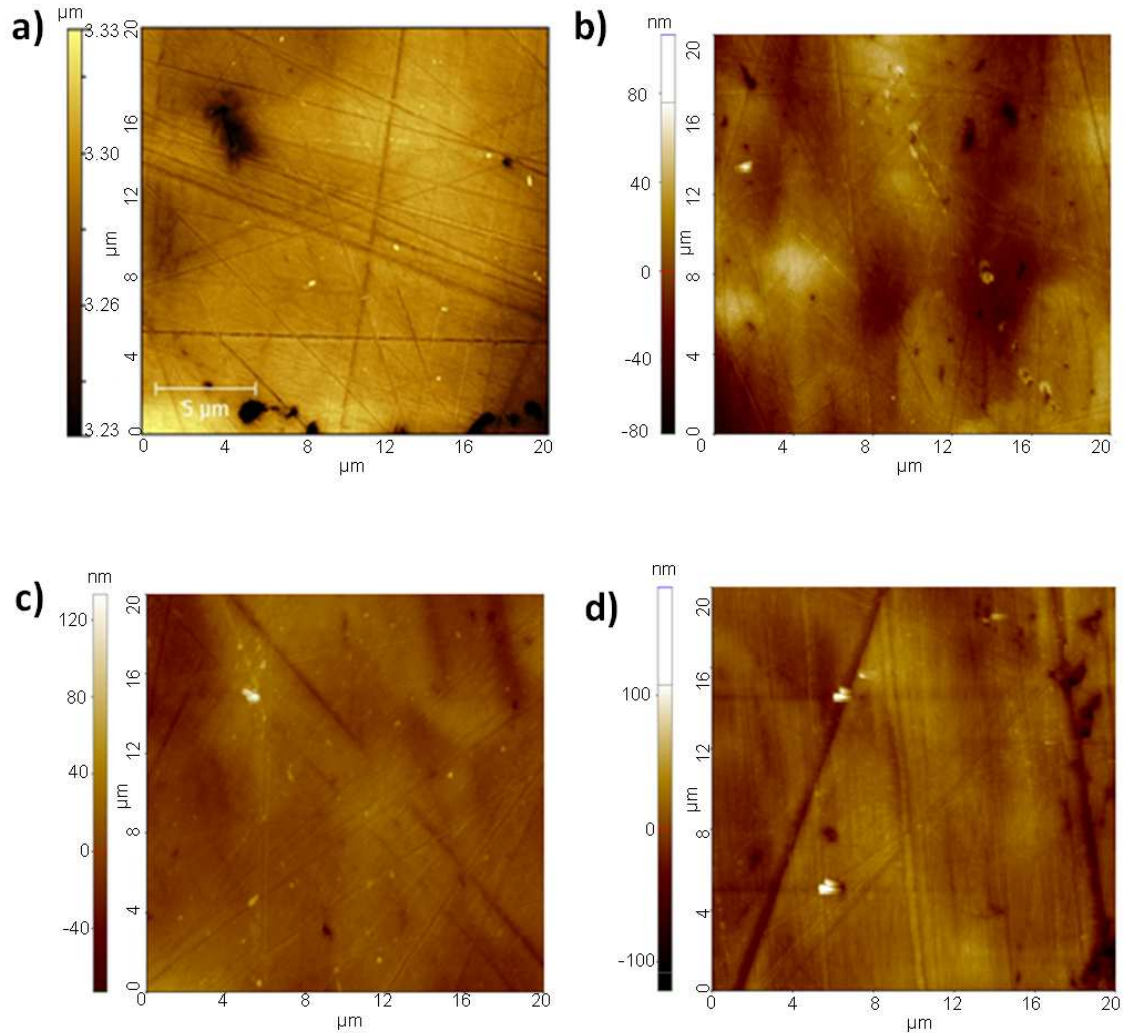


S4 Interaction of insulating PoAP with BocVGGVGOEt solutions: XPS C1s spectra (on the left) and AFM images (on the right) of the Pt/PoAP electrodes exposed to fresh (a), 15 days (b) and 76 days (c) aged VGGVG solutions.



S5 XPS C1s spectra (on the left) and AFM images (on the right) of the detection of (VGGVG)₃ employing the Pt/PoAP/BocVGGVGOEt electrodes obtained with fresh (a), 15 days (b) and 76 days (c) aged monomer solutions.

Role of Platinum substrate



S6 AFM images of Pt in its pristine state (a) and after interaction with BocVGGVGOEt fresh (b); 15 days (c) and 76 days (d) aged solutions. **The sequence of experiments is to be compared with its analogue in S4.**