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Binding and 2D organization of arginine on Cu(110)

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Abstract

In this work we present a detailed surface science characterization of L-arginine adsorption on the Cu(110) surface in a submonolayer regime. Arginine (ARG) is one of the main components of the tripeptide RGD (arginine-glycine-aspartic acid) commonly used as a linker/binder when engineering biomedical devices such as integrin receptors. We replaced the traditional Knusden cell sublimation method to obtain molecular films by dosing arginine directly from an aqueous solution through an electrospray ionization device (ESI). X-ray Photoelectron Spectroscopy (XPS) evidenced the co-existence of different adsorbed molecular species. In addition, Scanning Tunneling Microscopy (STM) data show that the arginine molecules form short and well-separated lines, constituted of dimers of molecules on the surface. DFT calculations helped clarifying these experimental findings, bringing strong evidences that ARG molecules adsorb in an ionic and a neutral form, with varied binding modes between N atoms and Cu atoms from the surface. These different N-Cu bonds lead to the establishment of intermolecular H-bonds, responsible for the dimerization process.

KEYWORDS: Arginine, UHV, XPS, STM, DFT, amino acids, metal surfaces, electrospray.

1-Introduction

There has been a considerable interest for deciphering the adsorption mode and self-organization of simple amino acids (AA), like glycine and lysine for instance, or more complex ones, such as methionine or histidine, on metal single crystal surfaces [1-3]. These studies were mostly motivated by the remarkable ability of these chiral molecules to form chiral self-assemblies and supramolecular 2D networks, particularly on copper single crystal surfaces [4-8]. Amino acids have a strong tendency to adsorb at metal surface via the amino and acidic functions of the asymmetrical carbon, inducing at least three possible anchoring points via both O atoms and N atoms of the amine group. The anchoring mode will play a crucial role in the reactivity of the adsorbed species or in its ability to form 2D arrays. For instance, alanine molecules on Cu(110) adsorb, as a function of surface coverage, by only one O atom or by both O atoms and N atoms, changing at the same time the molecular orientation of the adsorbed species and the 2D chiral motifs [4, 5]. Besides that, one should not forget the crucial role of the nature of the lateral chain in the further 2D molecular arrangements. The best example known to date is the differences observed between glycine, alanine and lysine adsorbed on Cu(110). All three molecules adsorb in a 3-points binding via both O atoms and N atom, but the nature of the terminal group (-H, -CH₃ or -NH₂, respectively), induces drastic changes in the 2D arrays: a (3x2) unit cell for glycine [7], a (2 -2, 5 3) unit cell for alanine [4, 5] and a (-2 -2; 5 -1) unit cell for lysine [9]. Finally, another key parameter that influences the adsorption mode and subsequently the supramolecular assemblies of a given molecule is the chemical nature of the adsorbed species. We have shown recently that it is possible to tune adsorption mode, geometry and supramolecular assemblies of a given molecule by changing its deposition method: from the gas phase or from an aqueous solution [10-12]. The latter is achievable if an Electrospray Ionization (ESI) device is employed, instead of a traditional Knudsen cell. As described in details elsewhere [13-15], using an ESI device enables to introduce molecules into UHV conditions, while they are still surrounded by their solvation sphere. Besides, ESI maintains the chemical integrity of the molecules and permits keeping them in a chemical ϵ -state resembling the one they have in a biological, aqueous, natural environment. Such experimental conditions turned out to be essential in the study of arginine.

Arginine (ARG) is a non-toxic and biocompatible AA which, together with aspartic acid and glycine, constitutes RGD (arginine-glycine-aspartic acid), a highly complex tri-peptide, utilized to give biocompatible and adhesive properties to materials and also as a specific binder and linker for integrin receptors devices, for instance [16-18]. Studying adsorption of ARG is thus part of a project aiming at the comprehensive knowledge of the RGD adsorption mechanism. Adsorption of aspartic acid, which occupies the other end of RGD with respect to ARG, has been recently investigated on several copper surfaces, showing quite complex adsorption modes and 2D structures [19-22]. As for ARG which shows

great similarities with lysine or methionine, it is frequently employed in nanotechnologies as corrosion inhibitor [23] and metal nanoparticle stabilizer [24] and plays a potential key role in biological interfaces [25]. Notwithstanding, it has given rise to a very limited number of investigations, especially under vacuum conditions; this can be easily explained by the difficulty encountered in its sublimation. In fact ARG sublimation temperature (as high as 60°C) often induces molecule breaking. Conversely, the use of ESI, allowing the introduction in vacuum of a beam of molecular ions from a room temperature aqueous solution, is a more delicate and alternative way for UHV ARG deposition.

Bearing a guanidine chain, ARG (see its formula schematized in Figure 1) has two reactive chain ends, the carboxylic and unsaturated C=NH groups, which suggest a possible competition when interacting with a metal surface [23, 26-28]. This issue will be at the heart of the present paper and will be addressed by studying the behaviour of the molecule on Cu(110) surface. After obtaining a submonolayer coverage in UHV conditions thanks to an ESI device, we realized characterizations by XPS and STM. A first round of Density Functional Theory including dispersion (DFT-D) calculations have been also performed to help clarifying and deducing the most likely adsorption geometries of the molecules suggested by the complete set of our experimental data.

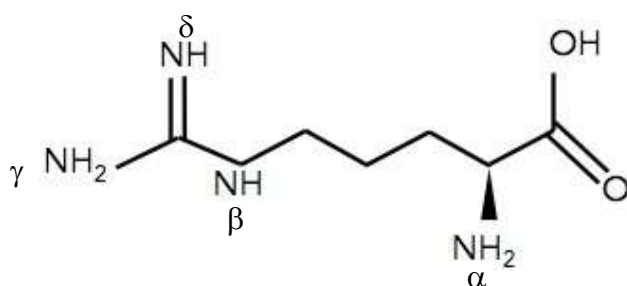


Figure 1. Schematic representation of ARG depicting the four different N atoms present in the molecule.

2-Experimental Details

All measurements were performed in UHV conditions, *i.e.* with a base pressure better than 1×10^{-10} mbar, with the sample kept at room temperature.

2.1-Materials.

Before Arginine (ARG) adsorption, the Cu(110) crystal (Surface Preparation Laboratory, The Netherlands, purity of 99.99% (4N) and alignment accuracies of 0.1°) was prepared for the experiments through several cycles of Ar⁺ sputtering (500 eV, $P_{\text{Ar}}=3 \times 10^{-6}$ mbar) and annealing at 850 K. XPS and LEED

(Low Energy Electron Diffraction) were used to check surface cleanliness and reconstruction. L-ARG was purchased by Sigma-Aldrich (99%) and used without further purification.

2.2-Dosing.

ARG adsorption has been realized from a 1 mM solution of ASP in ethanol/water 75:25 ratio by means of an ESI device. Explanations on ESI mode and principles of operation can be found elsewhere [10, 29]. Dosing of 2 minutes for XPS and 3 minutes for STM were used to obtain different ARG coverage. The pressure during the dosing varied in the range 1×10^{-7} to 5×10^{-7} mbar, with a base pressure of 5×10^{-8} mbar after opening the gate valve between the ESI vessel and the experimental chamber. A positive voltage of 1.5 kV was applied, guaranteeing a nominal ARG cationic gas phase state, *i.e.* COOH/NH₃⁺.

2.3-XPS.

Photoemission analysis was performed using a SPECS (Phoibos 100 1D Delay Line Detector) hemispherical analyzer and a monochromatized aluminum K_α X-ray source ($h\nu = 1486.6$ eV). The acquired spectra were calibrated according to the Cu Fermi level. The experimental uncertainty on the position in binding energy of the XPS peaks is 0.1 eV.

2.4-STM.

STM experiments were conducted in an Omicron Vakuumphysik XA VT-STM chamber with facilities for STM, LEED and sample cleaning. All STM images were acquired in constant current mode. The STM scanner was calibrated using the Cu(110)-O-(2×1) missing/added rows (interspacing distance = 0.51 nm).

2.5-Computational Details

The DFT calculations were performed using the Vienna Ab-initio Simulation Package (VASP 5.3) [30, 31], with projector-augmented wave (PAW) potentials [32, 33] with an energy cutoff (E_{cut}) of 400 eV. For each wavefunction the partial occupancies was set by Methfessel-Paxton's method at the 1st order and the width of the smearing was taken at 0.2 eV. The minimum number of electronic self consistent steps was taken at 4. The allowed error in the total energy is 10^{-4} eV and the break condition for the ionic relaxation loop is -10^{-2} eV. To better describe lateral intermolecular interactions between arginine molecules, we used the empirical dispersion correction of Grimme, known as D2[34]. The integration in the reciprocal space was performed with a Monkhorst-Pack grid[35]. The calculations of the supercell were performed at the Gamma point. We used the Perdew-Burke-Ernzerhof (PBE) functional[36]. The calculations were made using a Slab composed of 5 layers composed of 14 Cu each, the cells was also tilted by 15.80° in order to try to reproduce as well as possible the inclinations ($12^\circ \pm 3^\circ$ and $15^\circ \pm 4^\circ$) of Arginine lines observed on the copper surface. In preliminary calculations, we verified that the Cu atoms do not relax with adsorption, in agreement with STM data that reveal an unrelaxed Cu(110) surface. Therefore, to gain computational times, the Cu atoms were kept frozen.

Then, for the most stable structures, reoptimization in relaxing the Cu surface atoms confirmed that no surface relaxation occurs. Adsorption energies were computed as following: the adsorption energies was calculated as $E_{ads} = (E(\text{Slab-molecule(s)}) - nE(\text{molecule}) - E(\text{Slab}))/n$, where n is the number of molecules adsorbed (n = 1 or 2). The reference energies for the surface and the molecules were obtained by optimization of either the slab or the gas molecules in the same cell as studied for the adsorption, and the same calculation conditions.

The core levels were estimated at the initial state approximation, which has been shown to correctly reproduce the binding energies differences in N species adsorbed on Cu [37].

The absolute values of the core levels calculated significantly differ from the experimental values of the XPS binding energies. The reference binding energy was taken for the lowest binding energy computed, which corresponds to the δ -NH group, and has a binding energy of 397.5 eV. Typically, the core level at the initial state calculated for this moiety was 378.2 eV. We thus applied a systematic correction of the difference of these values to all the computed N 1s core levels. A correction of the Fermi level was not necessary as all computed Fermi levels falled at the same value. So, we could compare directly the binding energies computed for different configurations of adsorption. Thus, the correction was applied to all calculations performed for arginine adsorbed on Cu(110).

3-Results and Discussion

Figure 2 shows the O 1s and N 1s high resolution spectra of the Cu(110) surface after 2 min of exposure of ARG solution at $P = 10^{-8}$ mbar. The O 1s and N 1s peaks prove that ARG adsorbs on the investigated surface. The estimated average thickness of the so-obtained ARG film is 0.3 nm, indicating a submonolayer coverage.

The O 1s spectrum, Figure 2(a), displays only one component, centred at a Binding Energy (BE) of 531.6 ± 0.1 eV, due to the contribution from O atoms in the carboxylate groups (COO^-) [19, 38]. This allows us to conclude that ARG molecules, once adsorbed on the surface, have deprotonated carboxylic acid groups.

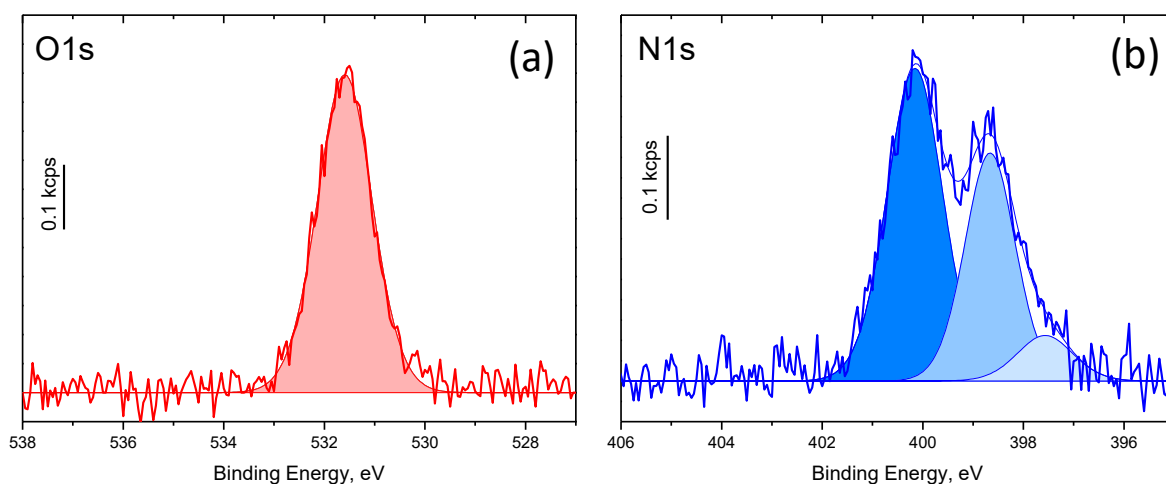
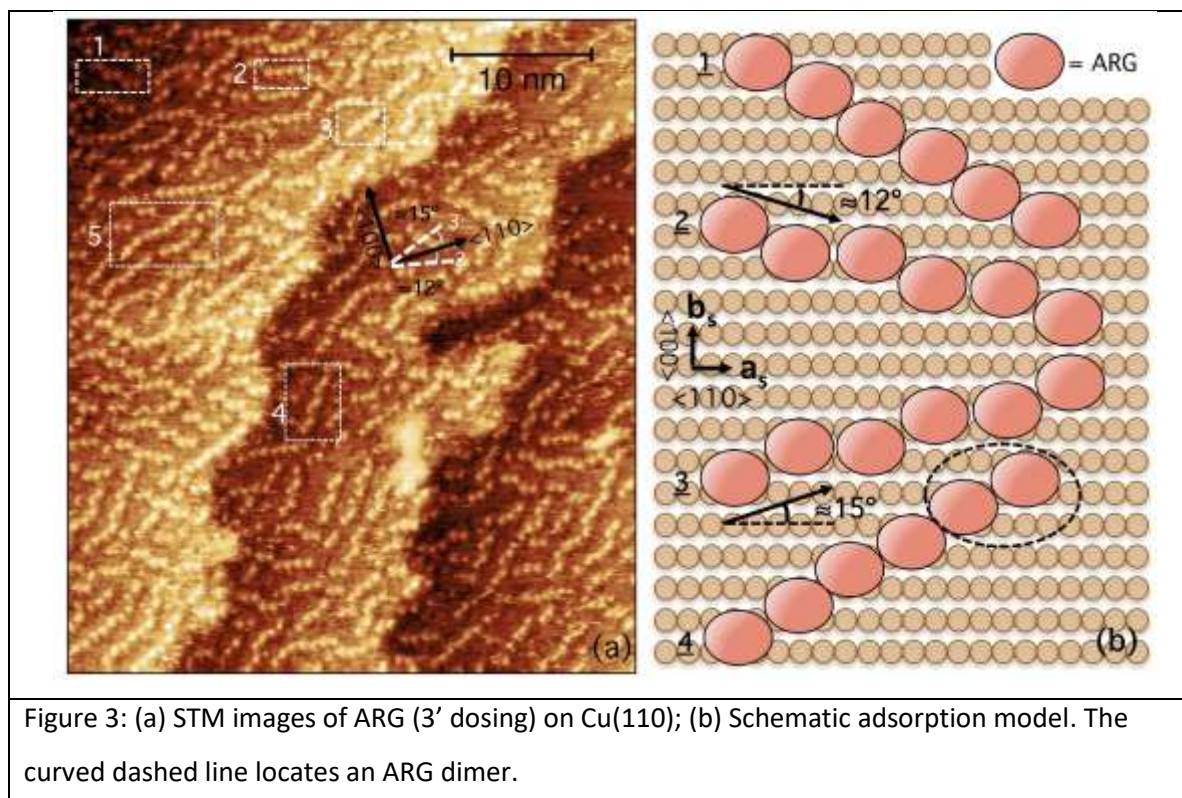


Figure 2. (a) O 1s and (b) N 1s spectra of ARG films obtained after a 2 minutes dosing on a Cu(110) surface.

The N 1s spectrum could be decomposed by a fitting analysis into three contributions, centred at BE of 397.6 ± 0.1 eV, 398.7 ± 0.1 eV and 400.0 ± 0.1 eV, respectively. Interestingly, there are no components with BE higher than 400 eV, which is instead the case when NH_3^+ contributions are found^{12, [39, 40]}. This indicates that no protonated $\alpha\text{-NH}_2$ are present on the surface. Apart from this immediate observation, attribution of each of the found components remains a difficult task. As a matter of fact, by simply looking at the component intensities, it appears that the N 1s spectrum indicates a non-stoichiometric ratio among the 4 ARG N atoms; thus various ARG species must be simultaneously adsorbed to give the observed line shape. Besides, in order to find an explanation for the obtained spectrum, several factors, either cooperative or competitive, have to be taken into account, such as the degree of the N(H) moiety protonation [38], the presence of inter and intramolecular H bonds, and the interaction with the surface (adsorption on a Cu atom).

To address this issue, we performed first an STM characterization of the adsorbed layer of ARG on Cu(110). STM image of ARG on Cu(110) after a 3 minutes dosing (Figure 3) shows an equally puzzling and, at a first look, random pattern, with no long-range order. At a more careful examination, we notice that all the structures on the surface are originated by the repetition of the same feature made of two brighter dots (square 1). This feature can be found aligned along two main directions (squares 2 and 3), forming angles of $12^\circ \pm 3^\circ$ and $15^\circ \pm 4^\circ$ (same angles within the experimental uncertainty) on the two sides of the $\langle 110 \rangle$ crystallographic axis. Moreover, these dots are assembled in different ways, giving rise to straight lines (squares 2 and 3), broken lines (squares 1 and 4) and combinations of these two structures (square 5). The measured unit dimensions allowed identifying the bright dots as two ARG molecules, probably in a dimer configuration and with their skeleton parallel to Cu rows. Thus,

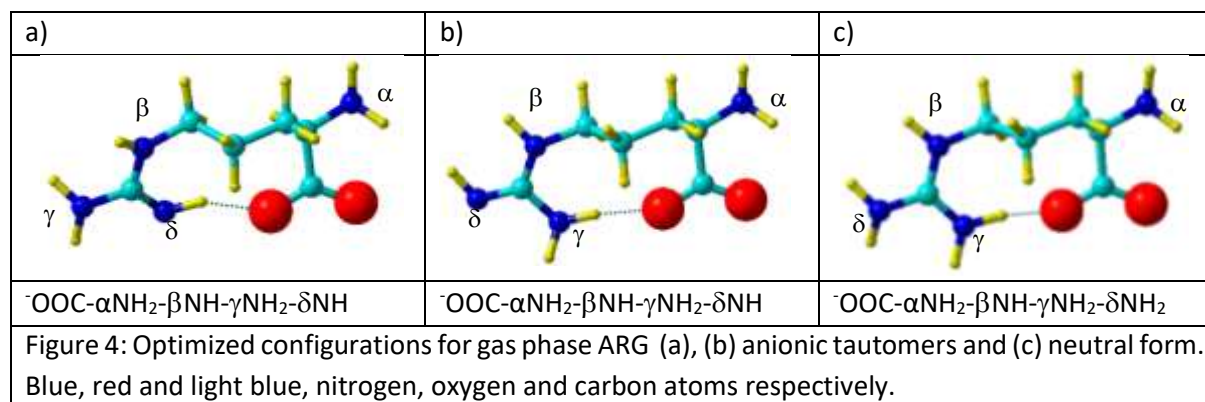
we propose the scheme reported in Figure 3(b) for ARG adsorption on Cu(110), where each red ellipse represents an ARG molecule.



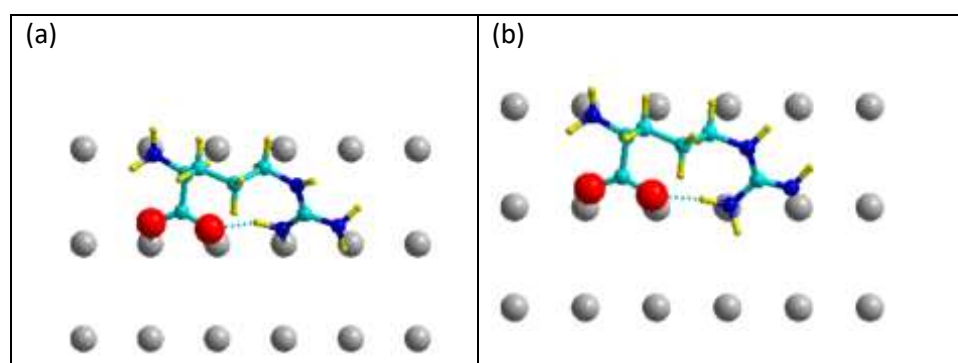
We thus went forward, realizing a DFT study of the ARG molecule/ion in gas phase as well as adsorbed on the Cu(110) surface, based on the surface molecular arrangements observed with STM and on the ARG chemical state deduced from XPS. In the choice of DFT initial configuration, a valid help came from some NEXAFS measurements performed on the O K-edge (see Figure AC1, in Associated Content section). These results clearly show that OCO planes containing the carboxylate ending of the molecule are oriented along the $\langle 110 \rangle$ direction. Nevertheless, since no certain results were available about the chemical state and the adsorption mode of the lateral chain, assumptions had to be made, especially regarding the δ/γ -NH₂ ends. Thus, after a meticulous examination of all possible gas phase configurations, three conformers emerged as the most likely for ARG molecule, two anionic (Figure 4(a) and (b)) and one neutral (Figure 4(c)). All of them are characterized by the presence of intramolecular H bonds between the lateral chain and the COO- group. The two ionic conformers differ for the function, NH or NH₂, in H-bond with the COO-moiety. The neutral form is a zwitterionic conformer that has been demonstrated by DFT to be as stable as the canonical form in the gas phase[41].

Afterwards, their interaction with the Cu(110) surface was examined. Figure 5 and Table 1 show the most stable configurations obtained and summarize the results. We found out that, whatever the

chemical state of the lateral chain, the molecule adsorption occurs through COO^- and $\alpha\text{-NH}_2$ moieties in a “glycine-like” configuration, as found for other amino acids.[8, 9, 42, 43]



Furthermore, the most stable anionic configuration occurs for the conformers of Figure 4(b), when H-bonds are established between the $\gamma\text{-NH}_2$ moiety and the adsorbed COO^- (Figure 5(b)). A strong bond is formed between one Cu surface atom and the $\delta\text{-NH}$ of the lateral chain with a N-Cu bond length distance of 2.08 Å. In contrast, a Cu-HNH bond is formed only when the $\gamma\text{-NH}_2$ end is H-bond donor to the COO^- moiety. We indeed notice, when comparing the two ionic configurations, that the HNH--O donor-like character of the bond (corresponding to a partial loss of the proton) is compensated by a reinforcement of the $\text{H}_2\text{N-Cu}$ interaction, the N-Cu distance evolving from 2.93 Å (when NH_2 is not in a donor H-bond) to 2.09 Å (NH_2 in donor H-bond), with the partial loss of the proton. This makes clear an interesting fact, i.e, the $\gamma\text{-NH}_2$ moiety has no affinity with the Cu surface, unless it is in a donor H-bond (and thus one H-N bond is weakened).



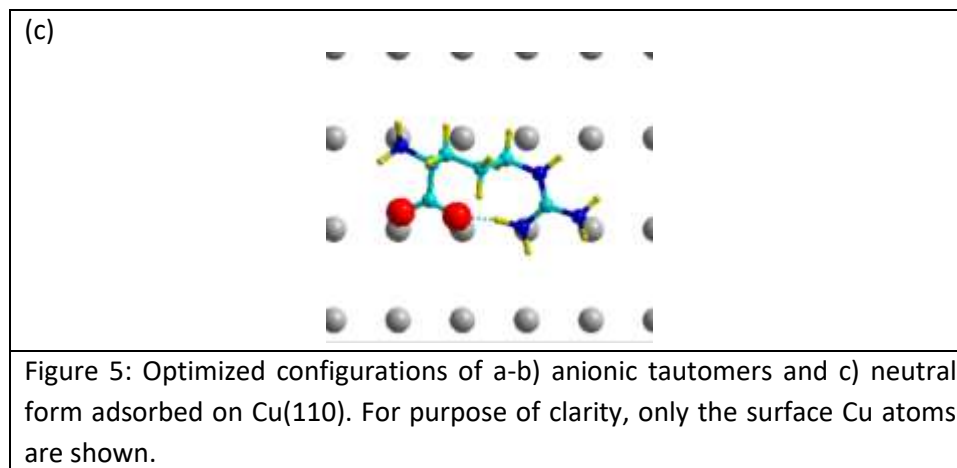


Table 1: Results of the geometry optimizations of ARG monomers on Cu(110)

Arginine chemical form	Number of H atoms	Adsorption mode	Relative energy ΔE (eV)	CLS calculations Δ_{CLS} (eV)
Anion (Figure 5(a)) α -NH ₂ β -NH, γ -NH ₂ , δ -NH	13	α -N-Cu : 2.15 Å γ -N-Cu : 2.93 Å δ -N-Cu : 1.98 Å and H-bond to O (1.84 Å)	0.15	
Anion (Figure 5(b)) α -NH ₂ β -NH, γ -NH ₂ , δ -NH	13	α -N-Cu : 2.20 Å γ -N-Cu : 2.09 Å and H-bond to O (1.91 Å) δ -N-Cu : 2.08 Å	0	δ -NH ₂ 397.6 α -NH ₂ 398.3 γ -NH ₂ 398.8 β -NH 399.0
Neutral form (Figure 5(c)) α -NH ₂ β -NH, γ -NH ₂ , δ -NH ₂	14	α -N-Cu : 2.15 Å γ -N-Cu : 2.62 Å and H-bond to O (1.68 Å) γ -N-Cu : 3.63 Å		α -NH ₂ 398.6 γ -NH ₂ 399.35 β -NH 399.8

For the neutral form, only one configuration is possible, shown in Figure 5(c). In this configuration, the bond between the lateral γ -NH₂ ends and the surface is weak (with N-Cu distances of 2.62 and 3.63 Å, respectively), indicating van der Waals interactions rather than chemical bond.

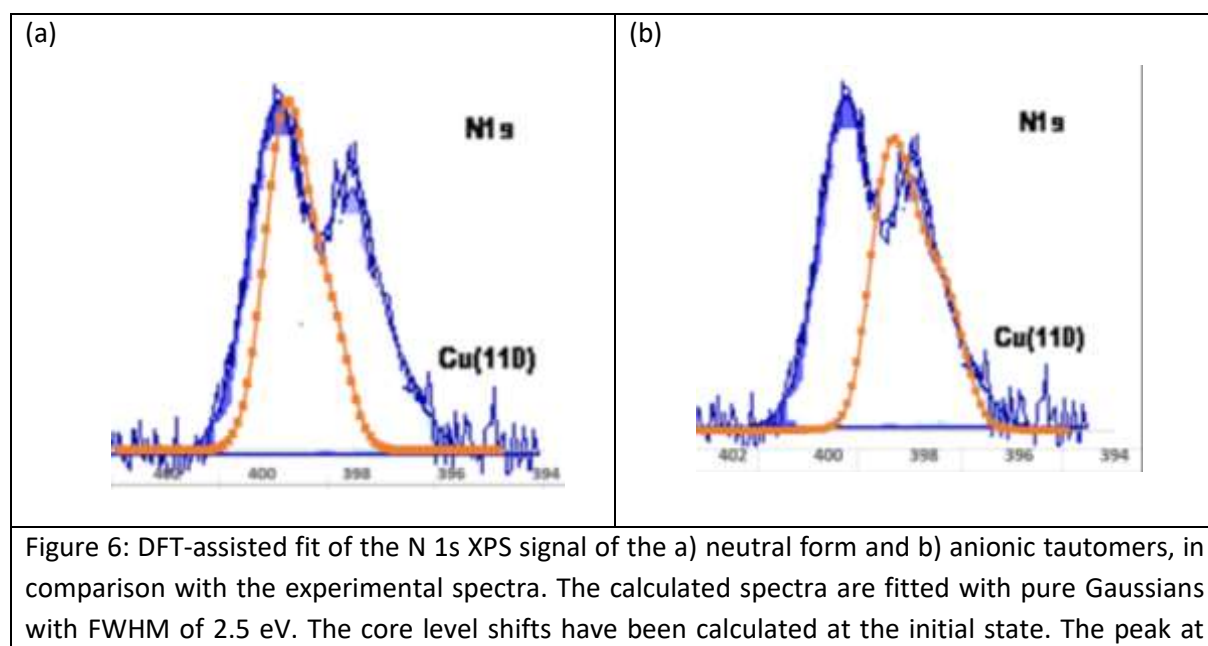
Note that the β -N end, in any of the studied configurations leads to a N-Cu bond, indicating a β -NH moiety not in interaction with the surface.

To summarize, the isolated ARG molecule adsorbs on the Cu(110) surface in a flat-like orientation, binding in a glycine-like manner (COO⁻-Cu and α -NH₂-Cu). The ionic conformers are characterized by an additional chemical bond when the lateral end contains a δ -NH end and, possibly, an additional Cu-NH₂ bond when the γ -NH₂ end is in a donor H-bond with COO⁻. The neutral form-lateral chain/surface interaction is instead weaker, occurring only via van der Waals interactions, as the bond lengths seem suggesting.

Let us now examine the calculated core level shifts (CLS), corresponding to the most stable anionic tautomer configuration and to the neutral configuration. They are reported in Table 1 and Figure 6 in comparison with experimental spectra. In the anionic state, the adsorption of the δ -NH moiety has the lowest BE (397.6 eV), as expected because of the =NH double bond. We calculate that the α -NH₂ has a 0.7 eV higher BE (398.3 eV), and that the β - and γ - ends have, respectively, a 1.4 eV (399.0 eV) and 1.2 eV (398.8 eV) higher BE. The simulated spectrum, shown in Figure 6(b), agrees well with the component at low BE of the experimental spectrum. For the neutral form, not surprisingly, we observe peaks at higher BE: at 399.8 eV for the β - end, at 399.35 eV for the γ -end and a contribution at 398.8 eV, due to the α -end. Now, the simulated spectrum agrees well with the high BE components of the XPS experimental spectrum (Figure 6(a)).

It is worth observing that, for the adsorbed anion, the β -end does not exhibit a signal at 400 eV (with a Δ BE of 2.4 eV with respect to the δ -end). In the anionic species in gas phase, we also do not observe such a high Δ BE between β and δ ends: indeed Δ BE with respect to the δ -NH are 0.8, 1.44, and 1.03 eV for α , β and γ , respectively. Li *et al.*[44] have also calculated that β -NH BE considerably decreases (by c.a. 4.5 eV) from the neutral (BE around 405 eV) to the anionic form (BE around 400.5 eV) in gas phase.

To summarize, the study of the isolated molecule adsorbed on Cu(110) shows that the molecule adsorbs with the COO⁻ and α -NH₂ ends in a glycine-like configuration. The lateral chain originates a bond with the surface by the δ -NH and an intramolecular bond is formed between COO⁻ and a lateral NH group.



lowest BE, clearly attributed to the δ -NH species, has been placed at 397.6 eV. The positions of the other peaks have been shifted accordingly.

In the attempt of explaining ARG dimers observed in STM images Figure 3, we then investigated several configurations of adsorbed dimers. We first studied the interactions in the “anion-anion” and “neutral-neutral” dimers and found that the BE for the dimers are the same as for the monomers. This suggests that the possible formation of H bonds between the two molecules does not affect significantly the BE of the individual species. They are reported in the Associated Content section, Table AC1. For the “anion-anion” case, examination of the calculated CLS revealed that, as for the isolated anion, low BEs are calculated, in the case of adsorbed δ -NH species. For the “neutral-neutral” case, always in agreement with the results on the isolated molecule, there are peaks at high BE, whereas the low BE shoulder is lacking. Therefore, we can conclude that the dimers of either the anionic or neutral form configuration lead to CLS that are reminiscent of the respective monomer adsorption.

Finally from CLS calculations, we deduced that dimers need to have an anionic component with the δ -NH end in interaction with Cu, to account for the low BE signal, and a neutral contribution to account for the high BE calculated for the β -NH end. Moreover, the experimental ratio of the low BE peak to the total N 1s spectrum is 1/8, meaning that in dimers, which contain 8 N atoms, only one δ -NH is present. This led us to propose the “anion-neutral” form and to the decision of investigating several possible arrangements of the anion-neutral dimer.

In Figures 7(a) and (b), the most stable configuration obtained is presented. In the dimer, the COO^- are not adsorbed on the same Cu row, and the two molecules have the same orientation, which allows the formation of one intermolecular H bond between the β -NH and the neighbour COO^- . We also verified that the presented configuration is the most stable among other combinations (see examples in the Associated Content section, Figure AC2). The adsorption energy is -4.00 eV/molecule. It is decomposed into DFT contributions, -2.61 eV/molecule, attributed to the Cu-O and Cu-N bonds formation, and the Van der Waals contributions, -1.39 eV /molecule.

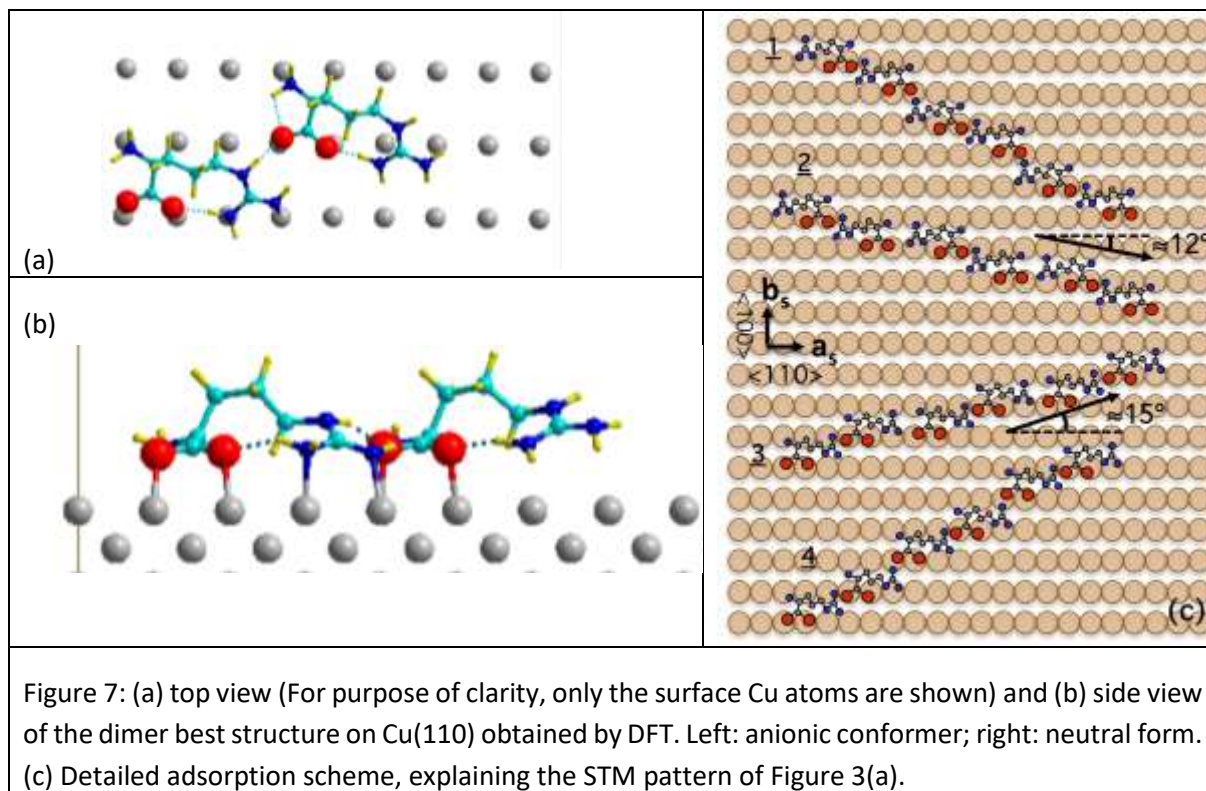


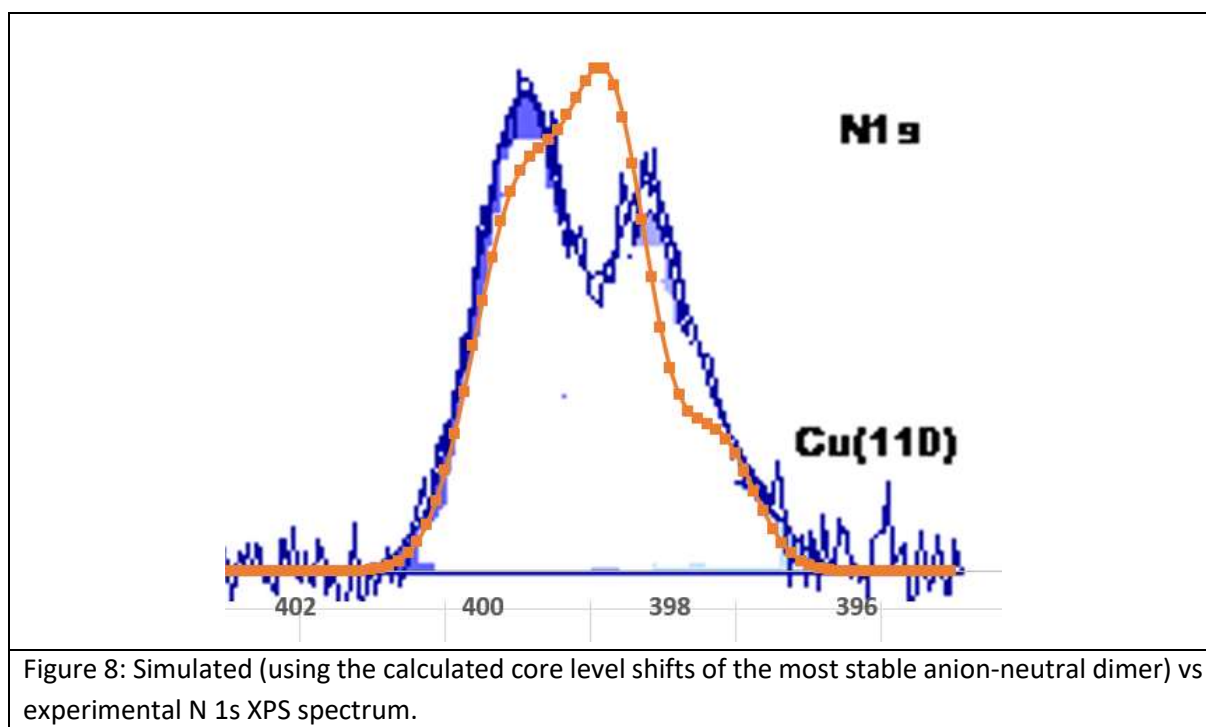
Table 2 reports the geometrical data and the calculated BE in the dimer. We observe that both molecules bind through the α -NH₂ to the Cu surface. The anion is adsorbed on the surface in a similar manner as when the molecule is isolated, bonded to the Cu surface via covalent bonds from the α -NH₂ and lateral NHs, at the exception of the β -end. The neutral form has also the same type of bonding with the surface as the isolated molecule, with formed N-Cu bond only through the glycine end, and the lateral N are now still farther from the surface: an intermolecular bond between the β -NH end of the anion and the COO⁻ end of the neutral molecule has formed; this was the last piece of the puzzle. By taking into account the obtained dimer most-likely structure, we were in the end able to resolve the molecular structure inside the red ellipses of Figure 3(b) and to propose an adsorption scheme for ARG molecules on Cu(110) (Figure 7(c)), reproducing some STM patterns of Figure 3(a). Note here that the situation where H-bonds are established between the β -NH end of the neutral molecule and the COO⁻ of the anion molecule, was also considered and is nearly isoenergetic (+0.03 eV difference, see Associated Content section; Figure AC2). Alternate, less stable configurations with other H-bonding possibilities are as well exposed in the Associated Content section, Figure AC2.

Table 2: Geometric details and calculated BE for the adsorbed dimer.

Molecule	N 1s Moiety	Cu-N distance (Å)	Calculated BE (eV)
anionic	α	2.20	398.30
	β	4.38	398.76

	γ	2.07	399.10
	δ	2.07	397.50
neutral	α	2.37	398.60
	β	4.88	400.08
	γ (intra H-bond)	3.81	399.60
	γ	3.03	399.67

Figure 8 reports the comparison between experimental and calculated XPS signals. Considering that the simulated species is an isolated dimer, and that the adsorbed dimers seen from STM have different possible orientations with respect to the surface, the obtained fit reasonably elucidates the chemical states of the molecules.



5-Conclusions

This work describes, to our knowledge, for the first time in the literature, a characterization of ARG adsorption on Cu(110), under ultra-high vacuum conditions. The single XPS O 1s peak, unambiguously shows the deprotonation of all COOH groups in the investigated submonolayer regime. However, STM and DFT calculations turned out to be necessary in order to explain the puzzling N 1s spectrum. STM shows that ARG molecules are spread rather uniformly and, at a first look randomly, on Cu(110). However, at a closer examination, linear features appear to be repeated several times on the surface, with various orientations, leading to structures with a specific design. These features were later

identified as being groups of 2, 3 or 4 ARG dimers. DFT calculations enabled to make clear that molecules, in the dimers, have not the same degree of protonation, one being a neutral form, the second an anion, thus explaining the non-stoichiometric intensities of N 1s components. Furthermore, DFT calculations helped proposing a configuration for the adsorbed dimer, where one molecule binds via its COO⁻ and α -NH₂ groups and the δ -NH and γ -NH₂ ends, while the nearby molecule only binds to the surface via the COO⁻ and α -NH₂ groups, with the lateral chain forming H-bonds with the COO⁻ of the first molecule. The appearance of rows of different orientations may be attributable to the initial orientations of different dimers, which finally join one to another via H bonds. This study, following the recent results obtained from aspartic acid investigation, marks a further step forward towards a thorough knowledge of RGD adsorption on Cu surfaces.

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