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# Self-Assembly of Glutamic Acid and Serine on Au(111)

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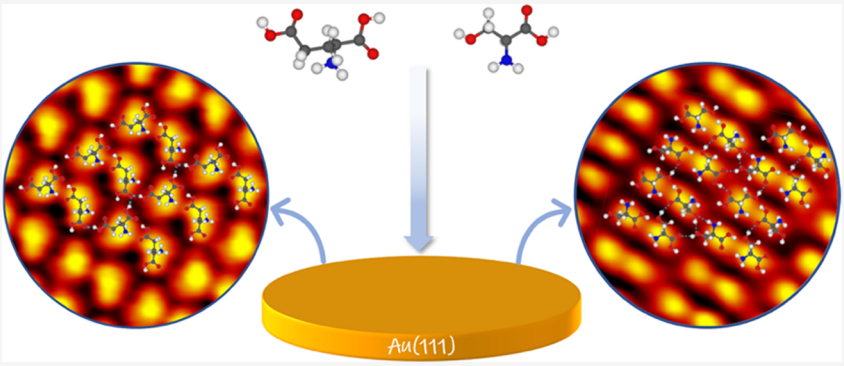


**ABSTRACT:** Amino acids provide novel and superior performance for two-dimensional materials and bio-based devices. The interaction and adsorption of amino acid molecules on substrates have thus attracted extensive research for exploring the driving forces involved in the formation of nanostructures. Nevertheless, the interactions within amino acid molecules on inert surfaces have not been fully understood. Herein, from the interplay of high-resolution scanning tunneling microscopy imaging and density functional theory calculations, we show the self-assembled structures of Glu and Ser molecules on Au(111), which are dominated by intermolecular hydrogen bonds, and further investigate their most stable structural models at the atomic scale. This study would be of fundamental importance in understanding the formation processes of biologically relevant nanostructures and provide possibilities for chemical modification.



## ■ INTRODUCTION

Biomolecules play an extensive role in many in vivo activities, which range from storing and expressing genetic information and maintaining normal metabolic functions to participating in various biochemical reactions.[1] Among them, amino acids, as the basic building block of proteins, can form a natural system of weak interactions via the intrinsic polar groups, i.e., amino groups ($-NH_2$) and carboxyl groups ($-COOH$). In the past decades, the adsorption and assembly of amino acid molecules have attracted broad interest from the field of surface science.[2] Accordingly, varieties of two-dimensional (2D) functional biomimetic materials have been fabricated at interfaces[3−5] due to their structural diversity and potential for artificial modification.[6,7] Based on the incorporation or functionalization of amino acid molecules, such as polymerizable histidine derivatives,[8] serine,[9] and glutamic acid,[10] biohybrid sensors have been successfully designed and exhibited desirable recovered toughness and great self-healing performance. Despite these efforts, among various kinds of amino acids, glutamic acid (Glu),[10,11] and serine (Ser),[12−14] playing important roles in protein metabolism in organisms, have also been widely studied in surface modification.[15,16] Moreover, surface analysis techniques, such as scanning tunneling microscopy (STM) and low-energy electron diffraction (LEED), provide a unique opportunity to directly observe nanoscale details of these self-assembled structures on surfaces.[17,18] It has been reported that Ser molecules adsorb on the copper surface in the anionic form,[17,19] while Glu molecules adsorb on the silver surface in the zwitterionic form and further change to anionic glutamate at relatively high coverage, during which the deprotonated oxygen atoms of the carboxyl groups intensively interact with the substrates.[20−23] In both cases, the self-assembled structures are dominated primarily by the strong interactions between the molecules and the underlying substrates. However, the self-assembled structures of Glu and Ser molecules on a relatively inert metal surface have been less investigated. It is, therefore, of utmost interest to study such self-assembled structures, which may facilitate the understanding of interactions between intrinsic amino acids and provide more possibilities for the prospective design of chemical modification.

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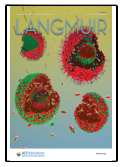




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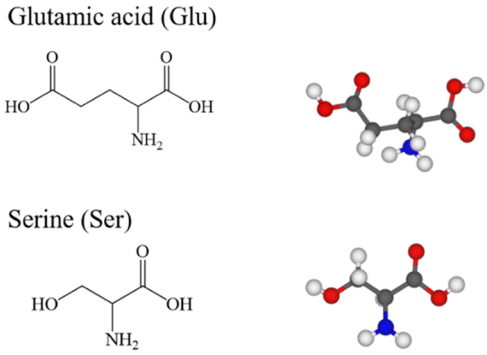
In this study, glutamic acid (Glu) and serine (Ser) molecules (cf. Scheme 1), together with a noble Au(111) surface, are chosen as a model system to explore the fundamental intermolecular interactions among amino acids. An inert Au(111) surface is employed as a support to weaken the influence of the substrate, on which the potential interactions among molecules could be explored. From the interplay of high-resolution STM imaging and density functional theory (DFT) calculations, we show the well-ordered self-assembled structures of Glu and Ser molecules on Au(111) stabilized by intermolecular hydrogen bonds, respectively. By carefully modeling the self-assembled structures, the Glu dimer and Ser dimer, which consist of molecules with opposite chiralities, are demonstrated to be elementary structures and further form racemic periodic structures. Our study demonstrates the formation of well-ordered self-assembled structures of Glu and Ser molecules at an atomic scale, which may further facilitate the exploration of fundamental mechanisms of adsorption and assembly of amino acids in real space.

**Scheme 1. Chemical Formulas and Gas-Phase-Optimized Structural Models of Glutamic Acid (Glu) and Serine (Ser) Molecules**




## ■ EXPERIMENTAL METHODS

All STM experiments were performed in a UHV chamber (base pressure $1 \times 10^{-10}$ mbar) equipped with a variable-temperature, fast-scanning "Aarhus-type" STM using electrochemically etched W tips purchased from SPECS,[24,25] a molecular evaporator, and other standard instrumentation for sample preparation. The Au(111) substrate was prepared by several cycles of 1.5 keV $Ar^+$ sputtering, followed by annealing to 800 K for 15 min, resulting in clean and flat terraces separated by monatomic steps. The glutamic acid and serine molecules (purchased from the Chinese Peptide Company, with a purity > 95%) were loaded into glass crucibles in the molecular evaporator. After thorough degassing, the molecules were deposited onto the Au(111) surface by thermal sublimation. The sample was thereafter transferred within the UHV chamber to the STM, where measurements were carried out at ∼150 K. Scanning conditions: It = 0.6−0.9 nA and Vt = −1700 − 1900 mV. All of the STM images were further smoothed to eliminate noises.

The calculations were performed in the framework of DFT using the Vienna Ab Initio Simulation Package (VASP).[26,27] The Perdew−Burke−Ernzerhof generalized gradient approximation (GGA) exchange−correlation functional was employed,[28] and van der Waals interactions were included using the dispersion-corrected DFT-D3 method of Grimme.[29] The atomic structures were relaxed using the conjugate gradient algorithm scheme as implemented in the VASP code until the forces on all unconstrained atoms were c0.03 eV/Å. The simulated STM images were obtained by the Hive program based on the Tersoff−Hamann method.[30,31]

## ■ RESULTS AND DISCUSSION

After the deposition of Glu molecules onto the Au(111) surface held at room temperature (RT), a well-ordered self-assembled structure formed, as shown in Figure 1a. The close-up STM image (Figure 1b) reveals that the structure is composed of Glu dimers, as depicted by the white contour. The observable herringbone reconstructions of the Au(111) surface under the monolayer of Glu molecules confirms that the molecule−substrate interaction can be neglected (Figure S1). Thus, to further investigate the intermolecular interactions within the self-assembled nanostructure, extensive DFT calculations on a series of gas-phase structures have been performed on the basis of experimental observations. As shown in Table 1, both homochiral and heterochiral Glu dimers (i.e., (*L*,*L*)-Glu and (*L*,*R*)-Glu) have been taken into consideration. The network consisting of heterochiral (*L*,*R*)-Glu dimer is calculated to be the energetically most favorable one (highlighted by the green shadow), indicating that the interaction within the Glu structure most likely results from intermolecular OH···O hydrogen bonds (depicted by green dashed lines) between neighboring Glu dimers.[22,23] As seen in the model superimposed on Figure 1b,c, we can identify that the Glu molecules bind to each other mainly via OH···O and NH···O hydrogen bonds within each dimer, and double OH···O hydrogen bonds between the dimer (depicted by green dashed lines). The lengths of the hydrogen bonds are indicated in Figure 1b,c, which are in good agreement with the length range in the literature.[32−35] Based on the structural model, the

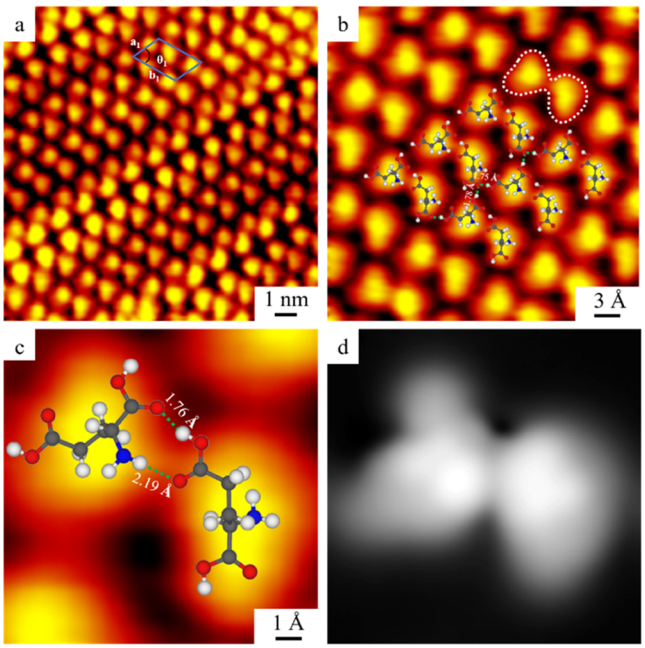



**Figure 1.** Self-assembly of Glu molecules on Au(111). (a) Large-scale STM image showing the well-ordered self-assembled structure of Glu molecules on Au(111). The indicated unit cell has parameters $a_1$ = 6.5 ± 0.1 Å, $b_1$ = 10.1 ± 0.1 Å, and $\theta_1$= 64 ± 1°. (b) Close-up STM image showing more details of individual Glu dimers (as typically depicted by the white contour). The superimposed DFT-optimized model shows the interconnections between Glu dimers. (c) High-resolution STM image of the Glu dimer superimposed with the DFT-optimized model. Hydrogen bonds are depicted by green dashed lines, together with the hydrogen bond lengths. H: white; C: gray; N: blue; O: red. (d) Simulated STM image of an isolated Glu dimer.


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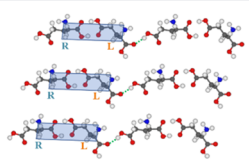
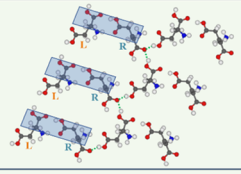
**Table 1. DFT Calculations on the Self-Assembled Structures of Glu Molecules and the Corresponding Total Energies of Different Networks**[a]

| | (L, L) - Glu dimer | (L, R) - Glu dimer |
|---|---|---|
| Network Structures of Glu molecules |  |  |
| Total energy (eV) | 1.56 | 0 |

[a]Energetically most favorable gas-phase structural model is highlighted.

simulated STM image of the (*L*,*R*)-Glu dimer (Figure 1d) is calculated, showing a good agreement with the experimental data.

As the next step, to extend the research model system, the adsorption and assembly of Ser molecules have also been explored on Au(111). Deposition of Ser molecules onto the Au(111) surface, followed by annealing at 400 K, leads to the formation of a well-ordered self-assembled structure, as shown in Figure 2a. The close-up STM image (Figure 2b) reveals that the structure is composed of peanut-shaped Ser dimers (depicted by yellow and blue contours). The herringbone reconstructions are also clearly observed under the assembly of Ser molecules (Figure S2), which validates further calculations in the gas phase. To further investigate the intermolecular interactions involved, extensive DFT calculations on gas-phase structures have been performed based on experimental observations. As shown in Table 2, both homochiral and heterochiral Ser dimers (i.e., (*L*,*L*)-Ser and (*L*,*R*)-Ser) have been considered as the elementary structural motif. The left two panels in Table 2 show the networks composed of the identical dimers ((*L*,*L*)-Ser or (*L*,*R*)-Ser), with total energies of 0.95 and 2.43 eV, respectively, whereas in either case, no hydrogen bonds exist between neighboring Ser dimers. Thereafter, Ser structures consisting of (*L*,*R*)-Ser with a different organizational chirality of whole dimer (indicated by blue and yellow parallelograms) have been calculated. The energetically most favorable structural model (highlighted by the green shadow) is composed of (*L*,*R*)-Ser dimers with different chirality (highlighted by blue and yellow parallelograms). These two kinds of self-assembled structures in the right two panels of Table 2 show comparable stabilities, while the second one does not agree with the STM morphology (with a zigzag interaction mode between two rows). Closer inspection of the network (overlaid with models in Figure 2b) shows that it is composed of heterochiral Ser dimers (depicted by blue and yellow contours) and the neighboring Ser dimers are linked via NH···O and OH···O hydrogen bonds (depicted by green dashed lines). From the high-resolution zoomed-in STM image and the structural model shown in Figure 2c, we attribute the unit cell to a Ser tetramer, in which the molecules in one dimer interact with each other via NH···O and OH···O hydrogen bonds (depicted by green dashed lines). The lengths of the hydrogen bonds are indicated in Figure 2b,c, which are in good agreement with the length range in the literature.[32−35] The corresponding simulated STM image of the (*L*,*R*)-Ser dimer (Figure 2d) shows a good agreement with the experimental data. From the above analysis, we conclude that the self-assembled structure of Ser molecules on Au(111) prefers to form the racemic framework.

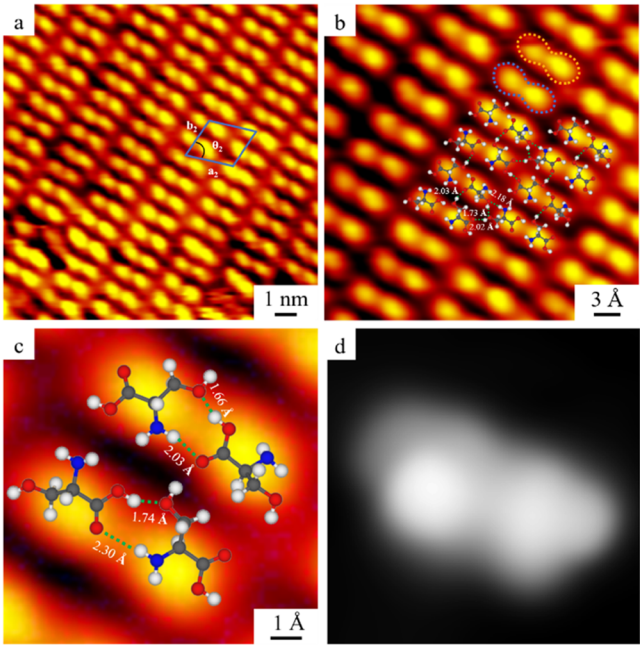



**Figure 2.** Self-assembly of Ser molecules on Au(111). (a) Large-scale STM image showing the well-ordered self-assembled structure of Ser molecules on Au(111). The indicated unit cell has parameters $a_2$ = 8.3 ± 0.1 Å, $b_2$ = 9.4 ± 0.1 Å, and $\theta_2$ = 68 ± 1°. (b) Close-up STM image superimposed with the energetically most favorable gas-phase structural model, showing the Ser dimers in different chiralities (depicted by yellow and blue contours). (c) High-resolution STM image of two heterochiral Ser dimers superimposed with the DFT-optimized model. Hydrogen bonds are depicted by green dashed lines, together with the hydrogen bond lengths. H: white; C: gray; N: blue; O: red. (d) Simulated STM image of the (*L*,*R*)-Ser dimer.

## ■ CONCLUSIONS

In conclusion, by the combination of STM imaging and DFT calculations, we have shown the real-space experimental observation of the self-assembly of two amino acids (Glu and Ser) via intermolecular hydrogen bonds on Au(111). Theoretical models show that the self-assembled structures of both Glu and Ser molecules prefer to form racemic structures. In contrast with previous studies, both Glu and Ser molecules are adsorbed on inert Au(111) in their neutral forms owing to the almost neglected molecule−substrate interactions, which allows the exploration of potential interactions among the amino acid molecules themselves. This study may help to


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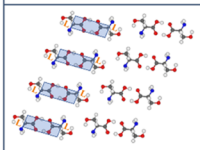
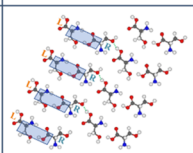
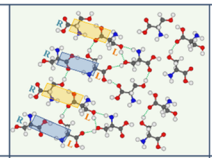
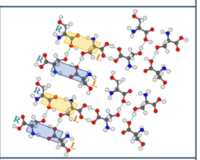
**Table 2. DFT Calculations on the Self-Assembled Structures of Ser Molecules and the Corresponding Total Energies of Different Networks**[a]

| | (L, L) - Ser dimer | (L, R) - Ser dimer | | |
|---|---|---|---|---|
| Network Structures of Ser molecules |  |  |  |  |
| Total energy (eV) | 0.95 | 2.43 | 0 | 0.09 |

[a]Energetically most favorable gas-phase structural model is highlighted.

extend a fundamental understanding of the self-assembly of amino acids governed by intermolecular interactions, which would further rationalize processes such as molecular recognition and chiral selectivity in biological systems.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.3c00055.

STM images showing the herringbone reconstructions of the Au(111) surface under the molecular monolayers of Glu and Ser molecules (PDF)

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### Author Contributions

All authors have given approval to the final version of the manuscript.


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### Notes

The authors declare no competing financial interest.


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## ■ ABBREVIATIONS

Glu, glutamic acid; Ser, serine; STM, scanning tunneling microscopy; DFT, density functional theory; UHV, ultrahigh vacuum

## ■ REFERENCES


(1) Dhanjai; Sinha, A.; Lu, X.; Wu, L.; Tan, D.; Li, Y.; Chen, J.; Jain, R. Voltammetric sensing of biomolecules at carbon based electrode interfaces: A review. *TrAC, Trends Anal. Chem.* **2018**, *98*, 174−189.

(2) Rahsepar, F. R.; Moghimi, N.; Leung, K. T. Surface-Mediated Hydrogen Bonding of Proteinogenic alpha-Amino Acids on Silicon. *Acc. Chem. Res.* **2016**, *49*, 942−951.

(3) Arroyo-Crespo, J. J.; Deladriere, C.; Nebot, V. J.; Charbonnier, D.; Masiá, E.; Paul, A.; James, C.; Armiñán, A.; Vicent, M. J. Anticancer Activity Driven by Drug Linker Modification in a Polyglutamic Acid-Based Combination-Drug Conjugate. *Adv. Funct. Mater.* **2018**, *28*, No. 1800931.

(4) Sloand, J. N.; Culp, T. E.; Wonderling, N. M.; Gomez, E. D.; Medina, S. H. Mechanomorphogenic Films Formed via Interfacial Assembly of Fluorinated Amino Acids. *Adv. Funct. Mater.* **2021**, *31*, No. 2104223.

(5) Castellanos Espinoza, R.; Sierra-Gómez, U.; Magdaleno López, C.; González-Gutiérrez, L. V.; Estela Castillo, B.; Luna Bárcenas, G.; Elizalde Peña, E. A.; Pérez Bueno, J. J.; Fernández Tavizón, S.; España Sánchez, B. L. Zwitterion-decorated graphene oxide nanosheets with aliphatic amino acids under specific pH conditions. *Appl. Surf. Sci.* **2021**, *555*, No. 149723.

(6) Ma, Q.; Song, J.; Wang, S.; Yang, J.; Guo, Y.; Dong, C. A general sensing strategy for detection of $Fe^{3+}$ by using amino acid-modified graphene quantum dots as fluorescent probe. *Appl. Surf. Sci.* **2016**, *389*, 995−1002.

(7) Guo, Y.; Nuermaimaiti, A.; Kjeldsen, N. D.; Gothelf, K. V.; Linderoth, T. R. Two-Dimensional Coordination Networks from Cyclic Dipeptides. *J. Am. Chem. Soc.* **2020**, *142*, 19814−19818.

(8) Özgür, E.; Parlak, O.; Beni, V.; Turner, A. P. F.; Uzun, L. Bioinspired design of a polymer-based biohybrid sensor interface. *Sens. Actuators, B* **2017**, *251*, 674−682.

(9) Guo, Q.; Zhang, X.; Zhao, F.; Song, Q.; Su, G.; Tan, Y.; Tao, Q.; Zhou, T.; Yu, Y.; Zhou, Z.; Lu, C. Protein-Inspired Self-Healable $Ti_3C_2$ MXenes/Rubber-Based Supramolecular Elastomer for Intelligent Sensing. *ACS Nano* **2020**, *14*, 2788−2797.

(10) Pei, H.; Wang, J.; Jin, X.; Zhang, X.; Liu, W.; Guo, R.; Liu, N.; Mo, Z. An electrochemical chiral sensor based on glutamic acid functionalized graphene-gold nanocomposites for chiral recognition of tryptophan enantiomers. *J. Electroanal. Chem.* **2022**, *913*, No. 116283.

(11) Han, Q.; Qiao, X.; Dongmei, G.; Can, L.; Yingzi, F. Chiral glutamic acid functionalized graphene: Preparation and application. *Anal. Methods* **2015**, *7*, 5387−5390.

(12) Kong, L.; Fan, D.; Zhou, L.; Wei, S. The influence of modified molecular (D/L-serine) chirality on the theragnostics of PAMAM-based nanomedicine for acute kidney injury. *J. Mater. Chem. B* **2021**, *9*, 9023−9030.

(13) Mathew, R.; Pujari-Palmer, M.; Guo, H.; Yu, Y.; Stevensson, B.; Engqvist, H.; Edén, M. Solid-State NMR Rationalizes the Bone-Adhesive Properties of Serine- and Phosphoserine-Bearing Calcium Phosphate Cements by Unveiling Their Organic/Inorganic Interface. *J. Phys. Chem. C* **2020**, *124*, 21512−21531.
(14) Tagirasa, R.; Yoo, E. Role of Serine Proteases at the Tumor-Stroma Interface. *Front. Immunol.* **2022**, *13*, No. 832418.
(15) Zhu, F.; Wang, J.; Xie, S.; Zhu, Y.; Wang, L.; Xu, J.; Liao, S.; Ren, J.; Liu, Q.; Yang, H.; Chen, X. l-Pyroglutamic Acid-Modified CdSe/ZnS Quantum Dots: A New Fluorescence-Responsive Chiral Sensing Platform for Stereospecific Molecular Recognition. *Anal. Chem.* **2020**, *92*, 12040−12048.
(16) Takahashi, M.; Yoshino, T.; Matsunaga, T. Surface modification of magnetic nanoparticles using asparagines-serine polypeptide designed to control interactions with cell surfaces. *Biomaterials* **2010**, *31*, 4952−4957.
(17) Iwai, H.; Emori, A.; Egawa, C. Self-Organization of L-Serine on Cu(001). *e-J. Surf. Sci. Nanotechnol.* **2009**, *7*, 821−824.
(18) Humblot, V.; Tielens, F.; Luque, N. B.; Hampartsoumian, H.; Methivier, C.; Pradier, C. M. Characterization of two-dimensional chiral self-assemblies L- and D-methionine on Au(111). *Langmuir* **2014**, *30*, 203−212.
(19) Eralp, T.; Shavorskiy, A.; Zheleva, Z. V.; Held, G.; Kalashnyk, N.; Ning, Y.; Linderoth, T. R. Global and local expression of chirality in serine on the Cu{110} surface. *Langmuir* **2010**, *26*, 18841−18851.
(20) Jones, T. E.; Urquhart, M. E.; Baddeley, C. J. An investigation of the influence of temperature on the adsorption of the chiral modifier, (S)-glutamic acid, on Ni{111}. *Surf. Sci.* **2005**, *587*, 69−77.
(21) Smerieri, M.; Vattuone, L.; Costa, D.; Tielens, F.; Savio, L. Self-assembly of (S)-glutamic acid on Ag(100): a combined LT-STM and ab initio investigation. *Langmuir* **2010**, *26*, 7208−7215.
(22) Jones, T. E.; Baddeley, C. J.; Gerbi, A.; Savio, L.; Rocca, M.; Vattuone, L. Molecular Ordering and Adsorbate Induced Faceting in the Ag{110}-(S)-Glutamic Acid System. *Langmuir* **2005**, *21*, 9468−9475.
(23) Smerieri, M.; Vattuone, L.; Kravchuk, T.; Costa, D.; Savio, L. (S)-glutamic acid on Ag(100): self-assembly in the nonzwitterionic form. *Langmuir* **2011**, *27*, 2393−2404.
(24) Besenbacher, F. Scanning tunnelling microscopy studies of metal surfaces. *Rep. Prog. Phys.* **1996**, *59*, 1737−1802.
(25) Laegsgaard, E.; Österlund, L.; Thostrup, P.; Rasmussen, P. B.; Stensgaard, I.; Besenbacher, F. A high-pressure scanning tunneling microscope. *Rev. Sci. Instrum.* **2001**, *72*, 3537−3542.
(26) Kresse, G.; Hafner, J. Ab initio molecular dynamics for open-shell transition metals. *Phys. Rev. B* **1993**, *48*, 13115−13118.
(27) Kresse, G.; Furthmüller, J. Efficient iterative schemes for ab initio total-energy calculations using a plane-wave basis set. *Phys. Rev. B* **1996**, *54*, No. 11169.
(28) Perdew, J. P.; Burke, K.; Ernzerhof, M. Generalized gradient approximation made simple. *Phys. Rev. Lett.* **1996**, *77*, No. 3865.
(29) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *J. Chem. Phys.* **2010**, *132*, No. 154104.
(30) Tersoff, J.; Hamann, D. R. Theory of the scanning tunneling microscope. *Phys. Rev. B* **1985**, *31*, No. 805.
(31) Vanpoucke, D. E. P.; Brocks, G. Formation of Pt-induced Ge atomic nanowires on Pt/Ge(001): A density functional theory study. *Phys. Rev. B* **2008**, *77*, No. 241308.
(32) Forster, M.; Dyer, M. S.; Persson, M.; Raval, R. Probing Conformers and Adsorption Footprints at the Single-Molecule Level in a Highly Organized Amino Acid Assembly of (S)-Proline on Cu(110). *J. Am. Chem. Soc.* **2009**, *131*, 10173−10181.
(33) Schiffrin, A.; Riemann, A.; Auwärter, W.; Pennec, Y.; Weber-Bargioni, A.; Cvetko, D.; Cossaro, A.; Morgante, A.; Barth, J. V. Zwitterionic self-assembly of L-methionine nanogratings on the Ag(111) surface. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5279−5284.
(34) Steiner, T. The Hydrogen Bond in the Solid State. *Angew. Chem., Int. Ed.* **2002**, *41*, 48−76.
(35) Xie, Y.; Liu, C.; Cheng, L.; Fan, Y.; Li, H.; Liu, W.; Zhu, L.; Li, X.; Deng, K.; Zeng, Q.; Han, S. Single molecular insight into steric effect on C-terminal amino acids with various hydrogen bonding sites. *Chin. Chem. Lett.* **2022**, *33*, 4649−4654.




https://doi.org/10.1021/acs.langmuir.3c00055
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