**Molecular Insight into** **the Interfacial Chemical Functionalities Regulating Heterogeneous Calcium-Arsenate Nucleation**

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**Abstract**

Heterogeneous nucleation induced by natural organic matter (NOM) can lower the energy barrier for calcium arsenate (Ca-As) precipitation, which aids in immobilizing arsenate (AsⅤ). However, it remains unclear how certain chemical functionalities of NOM affect Ca-As nucleation at the molecular scale. By analyzing changes in the local supersaturation and/or interfacial energy, the present work investigates the Ca-As heterogeneous nucleation kinetics and mechanisms on functional-group–modified model surfaces.

Mica surfaces modified by functional groups of amine (-NH2), hydroxyl (-OH), or carboxyl (-COOH) through self-assembled monolayers were used to investigate how chemical functionalities affect the Ca-As heterogeneous nucleation, in which the distributions of formation kinetics and size (as measured by the change in particle height) of nucleated Ca-As particles were measured by using *in situ* atomic force microscopy. In a parallel analysis, a quartz-crystal microbalance with dissipation was used to detect the buildup of Ca2+ and/or HAsO42− ions at the solid-fluid interface. PeakForce quantitative nanomechanical mapping and dynamic force spectroscopy using functional-group–modified tips made it possible to calculate the binding energies holding functional groups to Ca-As particles. Nucleated Ca-As particles were characterized by using Raman spectroscopy and high-resolution transmission electron microscopy.

The results indicate that the height of amorphous Ca-As particles formed on a modified mica surface may be ranked in descending order as –NH2 > –OH > bare mica > –COOH, as determined by the buildup of Ca2+ and HAsO42− ions at the solid-fluid interface and the decrease of interfacial energy due to the functional groups. These nanoscale observations and molecular-scale determinations improve our understanding of the roles played by chemical functionalities on NOM in immobilizing dissolved As through heterogeneous nucleation in soil and water.

**Keywords:** Interfacial chemical functionalities, Calcium arsenate, Heterogeneous nucleation, Self-assemble monolayer, Dynamic force spectroscopy.

1. **Introduction**

The addition of calcium-containing (Ca2+-containing) minerals such as lime (CaO) [1], calcite (CaCO3) [2], and brushite (CaHPO4·2H2O) [3] to arsenate-contaminated (AsⅤ-contaminated) soils and waters has proven to be a promising method to immobilize dissolved AsV. The mechanisms by which minerals immobilize AsV include surface adsorption [4,5], incorporations into the mineral lattice [6,7], and formation of calcium arsenate (Ca-As) through coupled dissolution-reprecipitation reactions at the mineral-water interface [2, 7]. Of these mechanisms, Ca-As precipitation is the main process for removing AsⅤ from contaminated soils and waters [8]. When calcium is added to AsV-containing solutions, Ca-As precipitates in with a relatively low water-solubility form, lowering the concentration and bioavailability of dissolved AsⅤ. Ca-As precipitates include CaHAsO4·*n*H2O (*K*sp = 10−4.68–10−4.79), Ca5H2(AsO4)4·9H2O (*K*sp = 10−30.69–10−31.49), Ca3(AsO4)2·*n*H2O (*K*sp = 10−18.91–10−21.40), Ca4(AsO4)2(OH)2·4H2O (*K*sp = 10−29.20), and Ca5(AsO4)OH (*K*sp = 10−40.12) [9–11]. Ca-As precipitates either through homogeneous nucleation in solutions or heterogeneous nucleation on substrate surfaces [12–14]. For homogeneous nucleation, the surface molecules of the nucleus are less strongly bonded to their neighbors than in the bulk solution, resulting in a higher energy barrier for nucleation [15]. In contrast, the presence of a substrate in heterogenous nucleation can lower the energy barrier by facilitating the formation of bonds with molecules in the nucleus, which leads to faster nucleation [16]. In addition, heterogeneously nucleated Ca-As phases are typically immobilized on the substrate, whereas the homogeneously nucleated phases remain suspended in the solution and are transported with the fluid before deposition, resulting in an increase in As-mobility [17].

Natural organic matter (NOM) is ubiquitous in soil and water environments and influences Ca-As heterogeneous nucleation [17, 18]. Previous bulk studies demonstrated that NOM forms complexes with Ca2+ and H*n*AsO4(3−*n*)− (where *n* depends on the local pH, see Fig. S1) [19–21]. However, NOM in the natural environment comprises different organic compounds with various functional groups [22], which limits the possibilities of molecular-scale investigation and thus hampers our understanding of the dynamics and mechanisms underlying Ca-As heterogeneous nucleation. The different components and structures of NOM are made of molecules with combinations of typical functional groups, including carboxyl (–COOH), methyl (–CH3), amino (–NH2), nitroso (–NO), hydroxyl (–OH), and sulfhydryl (–SH) [23–25], each of which leads to different interactions with Ca2+ and H*n*AsO4(3−*n*)−. Some interactions have been thoroughly investigated, such as covalent, coordinative, and electrostatic binding between the NOM functional groups and Ca2+ or H*n*AsO4(3−*n*)−, demonstrating that electrostatic attraction can be a significant force for NOM-Ca2+/ H*n*AsO4(3−*n*)− binding [26] through the most common functional groups found in NOM (i.e., –COO−, –OH, and –NH3+ at pH 7.0) [27]. However, how these functional groups affect Ca-As heterogeneous nucleation on the molecular scale remains unclear.

The present study uses the self-assembled monolayer (SAM) technique to investigate how surface-exposed functional groups affect Ca-As heterogeneous nucleation. First, we used atomic force microscopy (AFM) for direct, *in situ* observation on the nanoscale of the heterogeneous nucleation of Ca-As on groups –COOH-, –OH-, and –NH2-coated micas. Next, to further quantify the ion binding and heterogeneous nucleation of Ca-As at the SAM-fluid interface, we measured in real-time the adsorption of Ca2+, HAsO42−, and Ca-As on bare and SAM-coated sensors by using a quartz-crystal microbalance with dissipation (QCM-D). In addition, we modified AFM tips by bonding to them each of the three functional groups and quantitatively measured the adhesion forces and the binding energies between these model functional groups and the precipitated Ca-As by using PeakForce quantitative nanomechanical (QNM) and dynamic force spectroscopy (DFS) techniques. Finally, the phase and composition of the particles on SAM were characterized by using confocal Raman microspectroscopy and *ex situ* high-resolution transmission electron microscopy (HRTEM) coupled with energy-dispersive x-ray spectroscopy (EDX). These nanoscale observations and quantitative measurements improve our understanding of how the common functional groups in NOM affect Ca-As heterogeneous nucleation. These investigations provide insight into how NOM regulates the precipitation, growth, and transformation of Ca-As phases in soils and water.

1. **Materials and Methods**

**2.1 Direct observation of calcium-arsenate heterogeneous nucleation using *in situ* atomic force microscopy**

Freshly cleaved micas (Ted Pella Inc., Redding, CA, USA) were coated by using the SAM protocol of Deng *et al*. [28] with (3-triethoxysilyl)propyl succinic acid (J&K Scientific Ltd), silanol-terminated polydimethylsiloxane (Sigma-Aldrich, USA), and (3-aminopropyl)triethoxysilane (Sigma-Aldrich, USA) to obtain –COOH, –OH, and –NH2 functional groups on the mica surface, respectively. The modified mica was sealed in a 100 µL fluid cell on the AFM scanner (Multimode 8 AFM, Bruker). To avoid homogeneous nucleation of Ca-As in solution, we prepared fresh solutions for each experiment. A 100 mL freshly prepared Ca-As solution 3.81 mM CaCl2 (Sigma-Aldrich, USA), 3.81 mM Na2HAsO4 (Sigma-Aldrich, USA), and 132 mM NaCl (Sigma-Aldrich, USA); pH 7.0) were injected into the fluid cell at a constant rate of 10 mL/h by using a high-precision syringe pump. The constant rate of 10 mL/h was chosen to ensure steady-state kinetic nucleation rather than the diffusion-controlled nucleation during the experiments [29]. To track the heterogeneous nucleation of Ca-As phases on the various substrates, the coated micas were imaged continuously at a rate of 0.977 Hz with a resolution of 512 512 pixels using SNL-A tip with a spring constant of 0.35 N/m and a tip radius of 2 nm (as stated by the manufacturer). We used the measured particle height as the particle size (see Supporting Information).

* 1. **Assessing calcium-arsenate adsorption mass by using quartz-crystal microbalance with dissipation**

Gold QCM-D sensors (QSX-301, Biolin Scientific, Ltd., country) were used as substrate for self-assembled monolayers of alkyl-thiols with COOH [3-mercaptopropionic acid, HS(CH2)2COOH, Sigma-Aldrich, USA], NH2 [2-Mercapto­ethyl­amine hydrochloride, HS(CH2)2NH2·HCl, Sigma-Aldrich, USA], and OH-terminal [2-mercaptoethanol, HS(CH2)2OH, Sigma-Aldrich, USA] functional groups [30]. Before self-assembly, the sensors were cleaned as follows [31]: (1) exposure to UV radiation from an UV/Ozone cleaner (Bioforcy Nanoscience, country) for 10 min at 25 ℃; (2) immersion for 5 min at 75 ℃ into a mixed solution consisting of Milli-q water, ammonium hydroxide (25%, Bio-Lab, Ltd., country), and hydrogen peroxide (30%, Sigma-Aldrich, USA) at a volume ratio of 5:1:1; (3) washing three times with Milli-q water and twice with absolute ethanol (Sigma-Aldrich, USA); (4) drying with N2; and (5) exposure to UV radiation from the UV/Ozone cleaner for 10 min. Next, the activated sensors were immediately immersed into 1 mM alkyl-thiol ethanolic solutions with deoxygenation by Ar for 24 h at 25 ℃. Finally, the coated sensors were washed twice with ethanol and dried with N2. The self-assembled monolayers were characterized by using Fourier transform infrared (FTIR) microscopy (Vertex 70 with Hyperion-1000, Bruker, country) in reflection mode [32] and contact-angle measurements (OCA 20, DataPhysics Instruments, Germany) [33].

The real-time adsorption of Ca2+ or H*n*AsO4(3−*n*)− ions and heterogeneous nucleation of Ca-As particles in a Ca-As solution (3.81 mM CaCl2, 3.81 mM Na2HAsO4, and 132 mM NaCl) were monitored at 25 ℃ for 6 h by using an E4 QCM-D system (Qsense Biolin Scientific, Ltd., country). Before adding the solutions with CaCl2 and Na2HAsO4, a baseline was obtained by monitoring 132 mM NaCl for at least 12 h. Various frequencies (Δ*f*, Hz) were measured at overtones *n* = 3, 5, 7, 9, and 11. A higher overtone enhances sensitivity, but a lower overtone reduces interference by the solution. We used the *n* = 5 overtone in our measurements for further data analysis.

* 1. **Binding-energy measurement using Peakforce quantitative nanomechanical and dynamic force spectroscopy**

The binding energy was measured by using modified AMF tips. SNL tips (Bruker, Germany) were immersed into 3 mL 1% (wt, in methylbenzene) (3-triethoxysilyl)propyl succinic acid, silanol-terminated polydimethylsiloxane, and (3-aminopropyl)triethoxysilane solutions for 12 h each. The sensitivity to deflection error and the spring constant of each modified tip were calibrated at the beginning and end of each measurement. The tip modification was verified by comparing the rupture forces measured after coating with the rupture forces measured with a pristine tip (Fig. S2). Before each measurement, Ca-As particles were deposited on a freshly cleaved mica and then imaged by using the modified tip under the Peakforce QNM mode to obtain the surface topography and adhesion force simultaneously. During imaging, the Ca-As solution was injected into the cell at a constant rate of 5 mL/h to avoid dissolution of the Ca-As particles. The samples were imaged at a rate of 0.501 Hz and with a resolution of 128 × 128 pixels [34]. The DFS experiments were conducted by using the ramp mode with the modified SNL-D tips, following the calibration of the deflection-error sensitivity and spring constant. The tip approached the sample at a constant velocity of 200 nm/s and with a dwell time of 1 s both when approaching and moving away from the sample surface. To avoid contamination of the modified tip, a 2 nm deflection trigger point was used. A minimum of six retraction velocities, ranging from 20 to 3900 nm/s, were used for each measurement. To account for local heterogeneities, a minimum of 150 force curves were collected at various points for each retraction velocity, resulting in a minimum of 900 force curves for each measurement. The data were analyzed by using the Friddle-Noy-De Yoreo model [35]:

ln, (1)

, (2)

where is the equilibrium force, *k*B = 1.380649 10−23 J/K is Boltzmann’s constant, *T* = 298.15 K is the absolute temperature, is the transition distance between the unbound and bound states, = 0.577 is Euler’s constant, is the intrinsic unbinding rate, and is the binding energy. From each curve, we extracted the rupture force *f* and its corresponding loading rate *R*L. The fitting parameters from DFS data are listed in Table S1. IBM SPSS statistics software was used to analyze the data at P < 0.01.

**2.4 Particle identification with Raman spectroscopy and high-resolution transmission electron microscopy**

The Ca-As that precipitated following AFM imaging was further characterized by Raman spectroscopy (Horiba Jobin Yvon LabRam HR800, country) excited at 532 nm with the laser calibrated by using the 520.7 cm−1 band of silicon. To identify the phases, the precipitates (following the QCM-D measurements) were ultrasonically separated (Bandelin Sonorex, country) and characterized by HRTEM (JEOL, 2011) equipped with an EDX detector. All HRTEM images were acquired by using an acceleration voltage of 200 kV.

* 1. **PHREEQC simulations**

PHREEQC Interactive Version 3.3.7.11094 was used to model the Ca-As solution [36]. The simulations were performed by using the wateq4f database [9]. The solubility products *K*sp of the several As-containing phases not included in the database were obtained from the literature (Table S2).

1. **Results and Discussion**

**3.1 Direct observation of Ca-As heterogeneous nucleation on self-assembled monolayer**

Newly prepared micas with an atomically smooth (001) surface were used as a model mineral matrix with a high affinity for NOM [34]. Before injecting Ca/As mixed solutions, the bare and SAM-coated mica were characterized by Raman spectroscopy (Fig. S3) and AFM (Fig. S4). In contrast with the bare mica (Fig. S3A), new Raman peaks assigned to –COOH (Fig. S3B), –OH (Fig. S3C), and –NH2 (Fig. S3D) appear in the Raman spectra from the modified micas. The solution was then pumped into the fluid cell at a steady flow rate of 10 mL/h, and the nucleated Ca-As particles were observed as they formed heterogeneously on the surface (Fig. 1). The particle-height measurements can be used to unravel the thermodynamic origins of substrate-induced nucleation and the roles played by the functional groups in regulating the size of the nuclei. Figures 1 and S5–S8 show that the number of nuclei that form on each substrate increases with time. However, the particle height on all surfaces remains relatively constant (Figs. S5–S8), which indicates that nucleation rather than particle growth dominates the precipitation. Figure 1 also shows that the substrate-surface properties affect the average height of particles that form on the different surfaces. The average particle height (after reaction for 42 min) is 1.72 0.32 nm on –COOH-coated mica (Fig. 1B4), 3.07 0.42 nm on –OH-coated mica (Fig. 1C), 3.34 0.46 nm on bare mica (Fig. 1A4), and 9.25 2.88 nm on –NH2-coated mica (Fig. 1D).

The difference in particle height induced by various functional groups can be interpreted in terms of classical nucleation theory: a relatively small nucleus dissolves rather than grows [15, 37]. The critical nuclear radius (the nucleus is considered to be a sphere) is given by [13]

, (3)

where Ω is the volume per molecule (*r*Ca = 100 pm and *r*As = 46 pm), α is the interfacial free energy, and σ is the supersaturation, which is defined as [22]

σ = – 1, (4)

where *IAP* is the ionic activity product at the solid-liquid interface [*IAP* = (Ca)*n*(As)*m* for the reaction *n*Ca + *m*As → Ca*n*As*m*], and *ksp* isthe thermodynamic solubility product (the values for various Ca-As phases are listed in Table S2). In the present study, the critical size ranked in decreasing order is –NH2-coated mica > bare mica ≈ –OH-coated mica > –COOH-coated mica (Fig. 1F). Based on Eqs. (3) and (4), the critical radius of a nucleus decreases with increasing concentration of Ca2+ and H*n*AsO4(3−*n*)− at the interface and/or decreasing interfacial energy α, as discussed below.

**3.2 Using quartz-crystal microbalance with dissipation for real-time monitoring of adsorption of Ca2+, HAsO42−, and Ca-As on self-assembled monolayers**

The ion buildup caused by the attraction of Ca2+ and HAsO42− onto the modified QCM-D sensors (i.e., to the SAMs) was evaluated based on QCM-D measurements. The chemical composition of the gold surface following SAM deposition was confirmed by the appearance of new IR peaks due to the functional groups of the SAM (Fig. S9A) and by the changes in the contact angle of the SAM-coated sensors (Figs. S9B–S9E), which are consistent with reported values [31]. Figures 2A–2C present the the vibrational frequency F as a function of time after the addition of three solutions: CaCl2 (3.81 mM) in NaCl (132 mM), Na2HAsO4 (3.81 mM) in NaCl (132 mM), and a mixture of CaCl2 and Na2HAsO4 (3.81 mM each) in 132 mM NaCl (all at pH 7.0). A decrease in the frequency indicates an increase in sensor mass caused by the adsorption of ions onto the SAM. Because Na+ and Cl− were used as the background electrolyte and F remains constant after equilibrium is reached (Part Ⅰ in Fig. 2A), the mass increase on the sensor for the single-salt solutions (in NaCl) can only be due to the adsorption of Ca2+ and/or HnAsO4(3−n)−. The decreases in frequency following the addition of CaCl2 are 1.64 0.26, 3.99 1.34, 3.33 0.70, and 7.73 1.36 Hz for bare mica, –OH-, –NH2-, and –COOH-modified sensors, respectively (Fig. 2A). The adsorption of Ca2+ onto the –NH2-coated sensor may be attributed to the formation of the amine-Ca2+ complex [38, 39]. For HnAsO4(3−n)− (Fig. 2B), the adsorption capacity ranked in decreasing order is –NH2 (ΔFAs = 3.61 0.25 Hz) > –OH (ΔFAs = 2.68 0.17 Hz) > –COOH (ΔFAs = 1.63 0.36 Hz) > bare sensor (ΔFAs = 0.65 0.14 Hz). Interestingly, the mixed solution with CaCl2 and Na2HAsO4 produces a synergistic effect on the adsorption with the –COOH-coated sensor where the decrease of FCa-As (19.32 2.30 Hz) (Fig. 2C) exceeds the sum of the decrease for the single salts (ΔFCa and ΔFAs). Note that the synergistic effect occurred in all three substrates, especially for –COOH- and –NH2-coated sensors that attract Ca2+ and HAsO42−, respectively. These phenomena are attributed to the formation of Ca-As clusters with an adsorption capacity ranked in decreasing order of –COOH-modified sensor (ΔFCa-As = 19.32 2.30 Hz) > –NH2-modified sensor (ΔFCa-As =13.65 2.34 Hz) > –OH-modified sensor (ΔFCa-As = 7.69 1.94 Hz) > bare mica sensor (ΔFCa-As = 2.81 0.36 Hz).

The data from the QCM-D experiments show clear trends for the attraction of Ca2+ and HnAsO4(3−n)− that can be explained by electrostatic interactions: the buildup of Ca2+ ions is greatest for the COOH-coated sensor whereas the adsorption of HnAsO4(3−n)− is greatest for the positively charged –NH3+-coated surface (both at pH 7.0). In addition, the adsorption of ions on opposite charges is induced by electrostatic attraction, and the adsorption of Ca-As (for the mixed-ion solution) is attributed to the formation of hydrogen bonds (H bonds). In the present case, the strength of H-bonds may be ranked in descending order as –N–H···eAs > –O–H···eAs because O is more electronegative than N and limits the freedom of H in the formation of H bonds [40]. Weaker H bonds should correspond to less HnAsO4(3−n)− adsorbed on SAM-coated sensors. Figure 2 shows that, in contrast with ion adsorption, Ca-As adsorption is induced by both the negatively (–COO−) and positively (–NH3+) charged groups, which is similar to the effect of surface charge typically found on hydroxyapatite nucleation, which is similar to Ca-As particles [26]. For the –COOH-modified substrate, the concentration of Ca2+ increases at the substrate-fluid interface and excess positive charge attracts HnAsO4(3−n)− to form the –COO−–Ca2+–HnAsO4(3−n)− complex. For the –NH2-modified surface, the attraction of HnAsO4(3−n)− induces the formation of the Ca-As precursor phase or the [Ca*a*H*b*As*c*O*d*]*n*− ion cluster (where *a*, *b*, *c*, *d*, and *n* depend on its components), which is negatively charged.

**3.3 Energetic basis for functional-group–induced Ca-As heterogeneous nucleation**

In addition to the ions built-up at the solid-liquid interface, the interfacial energy plays an important role in regulating Ca-As heterogeneous nucleation. In the heterogeneous nucleation, the interfacial energy α comprises contributions from three interactions: the nucleus-liquid (αnl), nucleus-substrate (αns), and substrate-liquid (αsl) interactions, which are related as follows [41]:

α = αnl − *h*(αsl – αns), (5)

where *h* is a constant that depends on the relative surface area of the nucleus-substrate and substrate-solution interfaces. Given the lack of specific values of the interfacial energy for the nucleus-substrate-solution, α cannot be directly calculated [42]. However, the binding free energy Δ*G*b for any given element of a certain area *a* at the nucleus-substrate contact interface can be estimated based on the difference between the free energies in the nucleus-substrate-solution system, when the nucleus is bonded to the substrate. In this case, Δ*G*b is given by [41]

Δ*G*b = *a*(αnl + αsl – αns). (6)

Combining Eqs. (5) and (6), we derive the following relationship between Δ*G*b and α:

α = Δ*G*b + (1 + *h*) αns. (7)

In our heterogeneous precipitation experiments, we used the sample solution; that is, the values of αns remained consistent on the different substrates. Therefore, an increase in Δ*G*b between the nucleus and SAM substrate leads to a decrease in α as well as *r*c.

To measure Δ*G*b, AFM tips were decorated by the functional groups through Si-O-Si covalent bonding (Fig. 3A). The samples were imaged by using the decorated AFM tips in PeakForce QNM mode, which provides simultaneously the morphology (Fig. 3B) and adhesion force (Fig. 3C). Based on the force-separation curves (Fig. 3D), we deduced the rupture forces *f* between the functional groups on AFM tips and the Ca-As particles. We calculate *R*L from the corresponding force-time curves (Fig. 3D). According to Eq. (2), two fundamental regions exist: (1) a near-equilibrium region where *f* *f*eqwhen *R*L approaches 0, and (2) a kinetic region where we can derive *x*t. By fitting with the Friddle-Noy-DeYoreo model (Figs. 3E-G), Δ*G*b is calculated with the derived fitting parameters (Table S2). The result shows that the binding energies ranked in descending order give –COOH~Ca-As (14.98 1.37 kJ/mol) > –OH~Ca-As (10.18 0.92 kJ/mol) > –NH2~Ca-As (7.51 1.15 kJ/mol) > tip~Ca-As (4.74 0.37 kJ/mol) (Fig. 3H), which is consistent with the conclusion drawn from the particle height (Fig. 1F). This result is somewhat surprising when we consider that the surface of amorphous Ca-As is negatively charged. However, the results remain understandable because of the discrete nature of the amorphous surface, which allows functional groups to interact with the particles through Ca2+ sites despite the overall negative surface charge [43]. Moreover, the molecule bonded to the tip is forced to the particle surface, ensuring that the equilibrium bound state is achieved before rupture, regardless of the kinetic barrier to adsorption, and a net surface charge may appear. Therefore, the results of the Δ*G*b measurements are consistent with the general conclusion that a counterion-induced attraction plays a fundamental role in organic-mineral interactions. This result based on dynamics measurements demonstrates that the functional groups on NOM can decrease the surface free energy and thus promote Ca-As precipitation.

**3.4 Ca-As phase identification by Raman spectroscopy, high-resolution transmission electron microscopy, and PHREEQC calculations**

To characterize particles formed on the various substrates, the reacted samples were collected after AFM imaging and identified by using Raman spectroscopy. Two new Raman peaks appear at 862.4 and 1120.8 cm−1 from the Ca-As particles, corresponding to the *ν*1(AsO3) symmetric stretching mode and the δ As-OH in-plane bending vibrations (Fig. 1E) [44]. To further identify the phase, the precipitates were imaged by using HRTEM (Figs. 4 and S10). The corresponding fast Fourier transform patterns (Figs. S10A2–S10C2) confirm that the initial phases of these precipitates are amorphous Ca-As-Cl (Figs. S10A3–S10C3). These small amorphous particles can rapidly transform into weakly crystalline phases after electron-beam–induced coarsening (Fig. 4). Note that this transformation reduces the free energy of the system, regardless of whether it is exposed to electron beam irradiation or solution conditions [45]. However, insufficient electron-beam irradiation increases the local temperature and accelerates the transformation rate. Although we could not use the measured *d* value (Fig. 4B2) to identify the crystalline phases because of the lack of JCPDF cards for Ca-As-Cl and, based on PHREEQC calculations, all experimental solutions were supersaturated with respect to Ca5(AsO4)3Cl (Table S3).

1. **Conclusions**

Coprecipitation is a well-established method to decrease AsⅤ bioavailability in soil and water ecosystems, and heterogeneous nucleation plays a vital role in this process. Chemical functionalities on NOM can facilitate Ca-As heterogeneous nucleation. In the present study, we use the SAM technique to modify the mica substrate to accommodate –OH, –NH2, and –COOH functional groups and directly observe via *in situ* AFM the heterogeneous nucleation of Ca-As on bare and modified mica surfaces. The height of the particles formed on SAMs with the different surface functional groups are ranked in descending order as follows: –NH2 > bare mica ≈ –OH > –COOH. The differences in particle height are due to changes in the local Ca2+ and HAsO42− concentrations and surface free energies. Based on the decrease in resonance frequency (monitored by QCM-D), these functional groups in NOM are shown to attract Ca2+ and HAsO42− as well as Ca-As clusters from the bulk solution to the substrate-fluid interface. We also used modified AFM tips to measure the rupture forces and calculate the binding energies between Ca-As nuclei and the functional groups. The results demonstrate that –COOH has the largest binding energy and the smallest surface free energy of these functional groups.

This study provides a thermodynamic insight into Ca-As heterogeneous nucleation induced by functional groups on NOM, which can improve our understanding of the effectiveness of AsⅤ immobilization by precipitation. Furthermore, this study is not limited to Ca-As as precipitate but may be extended to the heterogeneous nucleation of Fe or Al-As precipitates [46–48] induced by NOM. Therefore, these nanoscale observations and molecular-scale determinations may deepen our understanding of the role played by NOM in immobilizing dissolved AsⅤ through metal cations in soil and water environments.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Supporting Information**

The Supporting Information provides equilibrium constants (Table S1), fitting parameters (Table S2), PhreeqC simulations (Table S3), As species distribution (Fig. S1), rupture-force distribution (Fig. S2), Raman spectra (Fig. S3), AFM height images (Fig. S4), time sequences of AFM images (Figs. S5–S8), IR spectra (Fig. S9), contact angles (Fig. S9), and HRTEM images (S10).

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**Fig. 1.** Ca-As heterogeneous nucleation at the interface of (A) bare mica, (B) –COOH-, (C) –OH-, and (D) –NH2-modified mica (001) surfaces in reacted solutions containing 3.81 mM CaCl2, 3.81 mM Na2HAsO4, and 132 mM NaCl at pH 7.0. The time sequence of AFM height images shows that the precipitation of a Ca-As phase occurs on the surface of (A1)–(A3) bare mica and (B1)–(B3) –COOH-modified mica with (A4), (B4) corresponding particle-height distributions after 42 min of reaction. AFM height images of (C) –OH- and (D) –NH2-modified mica (001) surface after 42 min of reaction. (E) The appearance of peaks at 1120.8 cm−1 (δ As-OH in-plane bending vibration) and 455.3 cm−1 [*ν*4 (AsO3) bending mode] in Raman spectra of mica after the reaction indicate that the nucleated particles contain Ca-As phases. (F) Particle height on various mica surfaces after 42 min of reaction. The uppercase letters (A–C) indicate significant difference at *P* < 0.01.



**Fig. 2.** QCM-D monitoring the adsorption of (A) Ca2+ and (B) HAsO42− ions and (C) the heterogeneous Ca-As nucleation on bare sensors (black), –OH-coated sensors (red), –NH2-coated sensors (blue), and –COOH-coated sensors (orange). Blue dashed lines divide the measurements into two parts: (I) a stable baseline for a 132 mM NaCl solution and (II) a region of decreasing frequency that starts at the injection of working solutions at 25 ℃. The decreases in frequency F indicate the adsorption of (A) calcium, (B) arsenate, and (C) the nucleus of calcium arsenate on the coating sensors. (D) The frequency shift ΔF after adsorption for 90 min of calcium and arsenate ions and heterogeneous nucleation for 210 min of calcium arsenate on bare sensors, Au sensors (yellow), –OH-coated sensors (green), –NH2-coated sensors (purple), and –COOH-coated sensors (orange).

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**Fig. 3.** Binding energy determined by using PeakForce quantitative nanomechanical and DFS techniques. (A) Schematic of DFS experimental system. Various functional groups, including –COOH, –OH, and –NH2, were covalently bonded to a Si3N4 tip through silanes reacting with silanol groups. (B) AFM height image of Ca-As particles on mica and (C) the corresponding adhesion-force distribution acquired with a –COOH-modified tip show that the adhesion force between –COOH and Ca-As particles is greater than that between –COOH and mica. (D) Representative force-separation curves and (E) corresponding force-time curve for extracting the rupture force and loading rate *r*L. DFS for determining equilibrium forces *f*eq, transition distances *x*t, and binding free energies Δ*G*b in reactions of (F) –COOH and Ca-As, (G) –OH and Ca-As, and (H) –NH2 and Ca-As. Solid lines in panels (F)–(H) are fits to the data using the Friddle-Noy-DeYoreo model [see Eq. (2) in Methods] [34]. (I) Experimentally derived values for the binding free energy showing the strongest binding pair of –COOH on Ca-As particles.



**Fig. 4.** Time sequence of HRTEM images of removedCa-As particles showing that (A) an amorphous particle can transform to (B) a crystalline particle upon electron-beam irradiation for 5 s. Panels (A2) and (B2) show the corresponding fast Fourier transform patterns taken from orange-dashed rectangular regions. The measured *d*-spacings are 0.18 and 0.21 nm in panel (B2).(C) EDX spectrum shows that the precipitates consist of O, As, Cl, and Ca.