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A Mesocrystal-Like Morphology Formed by Classical Polymer-Mediated Crystal Growth


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Growth by oriented assembly of nanoparticles is a widely reported phenomenon for many crystal systems. While often deduced through morphological analyses, direct evidence for this assembly behavior is limited and, in the calcium carbonate (CaCO₃) system, has recently been disputed. However, in the absence of a particle-based pathway, the mechanism responsible for the creation of the striking morphologies that appear to consist of subparticles is unclear. Therefore, in situ atomic force microscopy is used to investigate the growth of calcite crystals in solutions containing a polymer additive known for its ability to generate crystal morphologies associated with mesocrystal formation. It is shown that classical growth processes that begin with impurity pinning of atomic steps, leading to stabilization of new step directions, creation of pseudo-facets, and extreme surface roughening, can produce a microscale morphology previously attributed to nonclassical processes of crystal growth by particle assembly.

1. Introduction

Assembly of nanoparticles, molecular clusters, or other solution species that are more complex than simple ions, is now recognized as a common mechanism for crystal growth in a wide range of materials.[1] Dissolved macromolecules have been reported to drastically alter these pathways and their associated rates of formation. Examples—particularly with the use of acidic (poly)peptides[2] and proteins[3]—include the stabilization of amorphous precursor particles[4] (either solid[5] or liquid-like[6]), and the promotion of nanoparticle assembly with crystallographic coalignment to form so-called "mesocrystals"[1a,7] with exotic morphologies.[8] The concept of a mesocrystal was initially introduced to describe the coaligned assembly of calcite nanocrystals into a 3D kinetically stabilized superstructure in the presence of the organic additive polystyrene sulfonate (PSS).[7a] Since then many examples of mesocrystals have been reported.[7a,9] However, a recent critical reinvestigation of calcium carbonate mesocrystals grown with polymer additives similar to PSS showed there was no compelling evidence for the involvement of crystalline precursor particles in the formation of the apparent mesocrystal structure.[10] In fact, although observations of characteristic mesocrystal morphologies, rough nanoparticulate surfaces, high surface areas, and line broadening of powder X-ray diffraction patterns have commonly been considered indicators of a mesocrystal structure, the detailed analyses from that study led to the conclusion that the investigated calcite/PSS-MA (calcite/poly(4-styrene sulfonate co-maleic acid)) crystals did not fit the definition of a mesocrystal. Moreover, despite their outer mesocrystalline morphology, the crystals were demonstrated by transmission electron microscopy (TEM) imaging to have internal single crystal character. A strikingly similar morphology was also achieved through bulk overgrowth experiments on calcite single crystals.[10,11] Comparable exotic morphologies reminiscent of mesocrystals were
obtained for calcium carbonate in the absence of any additives though classical crystallization pathways. \[12\] Consequently, in spite of the numerous examples of mesocrystal formation via assembly of particles, both amorphous and crystalline, from biological as well as abiotic origin, \[1a\] the growth mechanism responsible for the creation of the exotic morphologies commonly attributed to mesocrystal structures in the absence of particle assembly is now unclear. Therefore, in this work we have used in situ atomic force microscopy (AFM) to investigate the overgrowth mechanism of calcite crystals utilizing PSS as the growth-modifying polymer. We show that the introduction of PSS does indeed lead to the formation of the exotic structures and morphologies, previously attributed to mesocrystal formation, but does so through stabilization of new step directions, coupled with step pinning that generates extreme roughening of the surface and broadening of crystal edges into pseudo-facets. Neither in situ AFM, cryo-TEM, nor light scattering data provides evidence for the attachment of nanoparticles of either amorphous or crystalline calcium carbonate to the growing crystal. Hence, we conclude that the final morphology arises through completely classical growth mechanisms.

2. Results

2.1. Bulk Diffusion Experiments

In our control benchtop experiments without PSS, after 1 day (1 d) of CO\(_2\) diffusion from the solid (NH\(_4\))\(_2\)CO\(_3\) source into a solution of \(1.25 \times 10^{-3}\) M CaCl\(_2\) within a desiccator, rhombohedral calcite crystals grew on a Si\(_3\)N\(_4\) substrate (Figure 1a) alongside a population of vaterite crystals. \[13\] In contrast, when PSS was introduced to the mineralizing solution, after 1 d of diffusion the typical rhombohedral morphology was modified through the flattening of the (001) face and the three adjacent crystal edges, resulting in a triangular (001) facet with a triradiate pattern of (018) facets (Figure 1b and Section 2.1, Supporting Information). The observed smaller size of the calcite-PSS crystals (14 ± 4 \(\mu\)m vs 48 ± 9 \(\mu\)m; average ± s.d. of the distribution) after 1 d of diffusion shows that calcite formation was significantly inhibited by the introduction of PSS (Figure S2, Supporting Information). By placing the Si\(_3\)N\(_4\) substrate perpendicular to the sedimentation direction in the diffusion setup, we ruled out sedimentation of the crystals from bulk solution and confirmed that the crystals nucleated and grew directly on the substrate (Figure S1, Supporting Information).

In all of the experiments, vaterite started to form at early diffusion time and, after longer time periods, spherical vaterite was still present next to the obtained calcite-PSS crystals as determined by Raman spectroscopy (Figure S3, Supporting Information). The initial formation of vaterite in the bulk diffusion experiments fixes the supersaturation for the calcite growth at the value given by the vaterite solubility product \(K_{sp, vat} = 1.2 \times 10^{-8}\) M\(^2\); see Section 2.2, Supporting Information). A depletion region was
observed surrounding the calcite-PSS crystals (Figure S5, Supporting Information) indicating that the vaterite likely dissolved and reprecipitated as the thermodynamically more stable calcite seeds grew. Despite the morphological changes and rough appearance of the (001) and (018) faces (which were determined by measuring angles in scanning electron microscopy (SEM) images; see Section 3), at this early stage of growth the (104) facets appeared surprisingly smooth (Figure S3, Supporting Information), and etching experiments in deionized water revealed dislocation etch pits on the (104) facets characteristic of single-crystal calcite (Figure S3, Supporting Information). However, the (001) plane dissolved more rapidly, and also the triradiate (018) faces etched more deeply than the (104) facets. We attribute these effects to: (1) a locally increased polymer content at these sites and\textsuperscript{[10,14]} (2) the higher surface energy of the (001) and (018) planes compared to the (104). Upon increasing the diffusion time (Figure 1c–e), the size of the (001) and (018) facets increased while the (104) facets became roughened, producing the overall shape and morphology previously considered to be an indicator of mesocrystal formation.\textsuperscript{[7a,14]}

Figure 1. SEM analysis showing development of calcite-PSS crystals grown in carbonate diffusion experiments at the microscale. a) Single crystal of calcite obtained in control (PSS-free) experiments (1.25 \( \cdot \) \( 10^{-3} \) M CaCl\(_2\)) after 1 d. b–e) Calcite-PSS crystals grown in solutions containing 1.25 \( \cdot \) \( 10^{-3} \) M CaCl\(_2\) and 0.5 g L\(^{-1}\) PSS after b) 1, c) 2, d) 3, and e) 5 d. d) indicates (018) pseudo-planar facets (black arrows) and the (001) pseudo-plane (orange arrow). f) Calcite-PSS crystal acquired from overgrowth experiment after 4 d (5 d including seed growth, see the Experimental Section), with indicated glide planes (yellow) and corners formed by convergence of either obtuse (+/+) or acute (−/−) steps in red. Scale bars: (a)–(c) 5 \( \mu \)m, (d)–(f) 10 \( \mu \)m.
The resemblance of the smooth (104) facets to those of single-crystal calcite motivated us to perform overgrowth experiments on single-crystal calcite seeds using the same diffusion method (similar to the experiments of Kim et al.,\textsuperscript{[10]} also see the Experimental Section). After diffusion times similar to those used in the nucleation and growth experiments, we found a remarkable resemblance in size, orientation, and morphology of the resulting crystals, with the (001) facet and adjacent (108) facets again expressed and the (104) faces highly roughened (Figure 1f, Section 2.1, Supporting Information). Confocal Raman spectroscopy confirmed that in the overgrowth experiments the end product was also calcite (Figure S3c,e, Supporting Information).

2.2. In Situ AFM Observation of Calcite Growth Modification by PSS at the Nanoscale

Although the experiments described above clearly demonstrate that the typical calcite-PSS crystal morphology observed in our nucleation and growth experiments can be obtained through overgrowth on preformed single-crystal calcite seeds (Figure 1), the final crystal morphology provides little information about the growth mechanism.\textsuperscript{[10]} Therefore, we used in situ AFM to study the overgrowth on the (104) face of a cleaved single crystal of calcite in a PSS-containing solution generated by mixing PSS-CaCl$_2$ and NaHCO$_3$ solutions at controlled pH (Experimental Section). The crystals exhibited growth hillocks formed at screw dislocations with discrete obtuse and acute steps typical for (104) calcite faces (Figure 2a). Step heights were determined to be 6.4 ± 0.6 Å (mean ± s.d. of the distribution, see also Figure S8, Supporting Information), in good agreement with the expected value for a screw dislocation having a Burgers vector $m = 2$ ($6.2$ Å, see ref. [15]). When PSS was introduced into the growth solution, obtuse and acute steps were progressively modified over time by step pinning, presumably due to the poisoning of kink sites (Figure 2b,c). Moreover, when comparing the growth hillock after PSS inflow (Figure 2b) with the one before (Figure 2a), we observe significant changes in the morphologies of both the obtuse and the acute steps, in terms of lateral dimensions, spacing, and height (Figure S8, Supporting Information). Most significantly, step pinning by PSS led to a vastly roughened expression of the acute steps with the protrusions of the roughened steps directed toward the facet boundaries. In addition, while the angle before PSS inflow between the two obtuse steps ([441] and [481]) had a value of $\approx 103.5^\circ$ (vs theoretical value of $101.9^\circ$, see ref. [16]), after its introduction, rounding of the obtuse steps led to a progressive increase in this angle to produce pseudo-[001] steps, again resulting in protrusions toward the facet boundaries.

We were also able to observe the step poisoning process in more detail (Figure 3a–c). In the presence of PSS, the measured 6.3 ± 0.5 Å height of the steps (Figure 3d–f) is in excellent agreement with the step height of 6.4 ± 0.6 Å determined without the added polyelectrolyte (Figure S8, Supporting Information). However, on the terraces between the steps, the fluctuations in height in the presence of the polymer are 7.0 ± 1.7 Å as compared to 2.4 ± 0.6 Å for the calcite surface in pure solution (Figure S8,
Supporting Information), with the difference attributable to adsorption of PSS molecules to the crystal surface\textsuperscript{[13,17]} (Figure 3d–f). The individual growth layer also exhibited the rounding of the obtuse steps and extreme roughening of the acute steps observed overall for the entire growth hillock.

Figure 2. Calcite growth modification by PSS at the nanoscale and its relation to creation of new facets. a) In situ AFM image of a calcite growth spiral obtained while flowing supersaturated PSS-free solution ($\sigma_{\text{calc}} = 2.53$) at 0.1 mL min\textsuperscript{−1} (see Materials and Methods, Supporting Information). The $c$-glide plane is indicated by the yellow dashed line. Acute and obtuse step directions are indicated with a minus and plus sign, respectively, in yellow. $+/+$ and $+/−$ indicate kinks moving toward the obtuse/obtuse and obtuse/acute corners of the growth hillock. b) In situ AFM image of the morphologically modified growth spiral obtained after flowing supersaturated solution ($\sigma_{\text{calc}} = 1.70$) containing 0.1 g L\textsuperscript{−1} PSS at 0.1 mL min\textsuperscript{−1}. c) Series of consecutive in situ AFM images (i–iv), for which influx of the PSS-containing solution begins between images (i) and (ii), showing modifications to obtuse and acute steps (i–iii). By image (iii), the stabilization of pseudo-[001] steps is evident and, by image (iv), the extreme pinning of the acute steps and roughening of the growth surface are shown. Scale bars in (a)–(c) are 500 nm. d–f) Schematic illustrating the geometry of the obtuse steps (in red, blue, and green) relative to the facet edges (dashed black lines) that form the [001] apex of the crystal d) before the PSS modification of growth hillocks, and e,f) after PSS modification. e) illustrates generation of the threefold pseudo-(001) facet through stabilization of the three [001] step directions and f) demonstrates the creation of the (018) family of pseudo-facets (black arrows) through convergence of the highly roughened steps.
2.3. Role of Amorphous Calcium Carbonate (ACC) in Calcite-PSS Crystal Formation

Given the extensive literature reporting the involvement of ACC during growth of calcite, an obvious question is whether there was direct involvement of ACC in the growth of the calcite-PSS crystals reported here. In the in situ AFM overgrowth experiments, the supersaturation with respect to ACC ($\sigma_{\text{ACC}}$) was calculated by determining the concentration of free Ca$^{2+}$ in solution ($c(\text{Ca}^{2+})_{\text{free}}$) using a calcium-ion-selective electrode (Ca-ISE). Here, the PSS partially complexes Ca$^{2+}$-ions in solution due to electrostatic interaction with the SO$_3^-$ groups.[13] We find that, on average, 0.25 ± 0.05 Ca$^{2+}$ are bound per SO$_3^-$, effectively lowering $c(\text{Ca}^{2+})_{\text{free}}$. However, the largest part of the total Ca$^{2+}$ added is present in form of ions in the bulk solution ($\approx 92\%$) (Figure S6a, Supporting Information). Consequently, we find that $\sigma_{\text{ACC}} = -3.11$, that is, the solution is heavily undersaturated with respect to ACC. This is in line with our AFM measurements, in which we do not observe growth on the calcite surface by addition of detectable ACC particles. Additionally, when investigating the growth solution by cryo-TEM and dynamic light scattering (DLS) we could not detect any ACC particles besides the presence of a minor quantity of small $\approx 6$–10 nm structures, which are presumably smaller Ca-PSS globules formed through complexing of Ca$^{2+}$ with negatively charged sulfonate groups of the PSS, in line with our previous findings[13] (Figure S6b,c, Supporting Information). Moreover, these particles show a low electron scattering contrast in brightfield TEM, as opposed to ACC.[18] Therefore, we conclude that the calcite crystals formed in the presence of PSS do not grow through the addition of amorphous CaCO$_3$ (see also Section 2.3, Supporting Information).

3. Discussion

Comparing the in situ AFM images to the SEM images of crystals from the benchtop diffusion experiments, we are now able to translate the modifications of growth hillock morphology from the nanoscale to the microscale, where the bulk calcite-PSS crystals exhibit roughened (104) faces bounded by roughened, threefold (001) faces and elongated (018) faces (Figure 1f). The data clearly show that the roughened (104) faces arise from pinning of the individual atomic steps and the threefold (001) faces are, in fact, pseudo-facets formed by pseudo-[001] steps created through polymer-poisoning of the obtuse steps. Because the facet boundaries lie in the six equivalent (018) planes, the convergence of the highly roughened steps from the hillocks on adjacent crystal faces produces a triradiate pattern of rough (018) pseudo-facets (Figure 2c,e,f). This effect is analogous to the broadening of the growth hillock boundaries created by convergence of the acute steps from adjacent hillock sectors (Figure 2b). The width of that boundary is of the same order of magnitude as the lateral undulations of the step fronts in the direction of the facet edges (Figure 2b,d–f). In the early stage of this process captured in
the AFM experiments, these step undulations are of order 1 µm, as is also the case for the (018) pseudo-facets during the early stages of formation in the diffusion experiments (compare Figure 1b and Figure 2b). The resulting crystal pseudo-facets lie at an angle of ≈27º with respect to the (001) plane, which, as expected, matches that of the (018) planes for which this angle has a theoretical value of 26.23º (see ref. [19]). Indeed, regarding external faces, we find a great resemblance of the calcite-PSS crystal with a simulated single crystal of calcite cleaved along the (001) and (018) planes (Figure S7, Supporting Information). Although the early stage of formation of the calcite-PSS crystals in the benchtop diffusion experiments differs from the AFM flow cell experiments, because there is no involvement of either ACC or vaterite in the latter, our study confirms that both the ammonium carbonate diffusion technique (including benchtop overgrowth experiments) and the mixing method produce a morphology previously attributed to “mesocrystal” formation. Moreover, the similarity of the outcome despite the differences in growth regimes further emphasizes the impurity effect of PSS on step growth. In the benchtop experiments, which rely on diffusion of carbonate into a solution with a fixed initial supply of Ca²⁺, the supersaturation drops as the crystals grow. At early times the supersaturation is high enough to drive nucleation of new crystals or 2D nucleation of islands on existing crystals, but in this regime the crystals are simple rhombohedra with smooth surfaces. In other words, there are no observed impurity effects despite the rapid growth. As the crystals grow and consume Ca²⁺, the supersaturation drops toward the regime explored by AFM, where the crystals grow more slowly (Figure S2, Supporting Information) and the “mesocrystal-like” morphology emerges. This is exactly what is expected from impurity pinning of steps, whether in a rough-step limit where the key factor determining the extent of pinning is the ratio of the critical step curvature to supersaturation, or in a smooth-step limit where the key factor determining the extent of pinning is the relative rates of polymer and solute binding to kinks.[20]

The growth mechanism revealed by these experiments clearly demonstrates the dominance of classical growth processes in producing the exotic crystal morphology with roughened patterns previously attributed to mesocrystal formation. However, the results do not directly reveal the atomic-scale mechanism that causes the acute steps to exhibit greater roughening and bunching than the obtuse steps, which indicates there is a greater degree of binding of PSS to the acute steps. There are two potential reasons for this difference. The first is simply that the configuration of the acute step results in better binding to the sulfonate groups of PSS. This mechanism is illustrated by the case of aspartic acid (Asp) on calcite for which even a switch from left-handed to right-handed Asp led to change in binding energy due to differences in the relative geometry of the Asp molecule and the mineral step edge.[21] These structure-dependent effects led to enantiomer-selective modification of the two acute steps and better overall binding of both enantiomers to the acute steps than to the obtuse steps. However, for larger molecules, a second mechanism associated with the energy removing waters of
hydration can play a significant role in determining differences in binding. For the peptide polyaspartate (Aspₙ), previous work found that molecules with three or more residues (ₙ ≥ 3) preferentially bound to the obtuse steps, because fewer water molecules needed to be removed. Thus, the dehydration energy was smaller for binding to the obtuse than the acute steps.[22] The number of water molecules released depends of course on the specific configuration of lowest energy and thus the effect should vary significantly from system to system. Thus, the same reasoning would imply that while PSS binds to both the obtuse and acute steps, the dehydration energy at the acute step is smaller than at the obtuse step.

Figure 3. Modification of a single calcite growth layer by PSS. AFM images of evolving growth steps in time at a) t = 0 s, b) t = 175 s, and c) t = 342 s. The yellow dashed line delineates the border of a single growth layer. A line profile is measured across the step (red dashed line) in the direction of the red arrow, and on top of the growth layer (blue dashed line) in the direction of the blue arrow. d–f) The corresponding height differences for images (a)–(c) along both lines are indicated in the graphs, respectively.

To understand the generation of the pseudo-[001] steps that define the (001) plane, we propose two possibilities. First, PSS exhibits a different binding affinity to the left versus the right facing kinks (Figure 2a), leading to a change in step shape. Maruyama et al.[23] found that the addition of l-aspartic acid during calcite growth led to different lengths for the originally symmetric [441] and [481] obtuse steps. They proposed that the effect could result from a difference in the resistance to incorporation into the two distinct kink-types—designated here as +/+ and +/− to indicate kinks moving toward the obtuse/obtuse and obtuse/acute corners of the growth hillock—due to kink blocking by the aspartic acid. A preferential poisoning of the +/+ kinks will
reduce the speed of the step toward that corner and thus lead to a flattening along the [001]. The second mechanism—perhaps acting in combination with the first—is related to the calcium density along the [001] step direction: As the shape of a newly formed step fluctuates during its advance, deformations toward the [001] direction will present an increased fraction of exposed Ca\(^{2+}\) sites and a net positive charge (see Figure 4). Therefore, complexing of the negatively charged sulfonate groups of PSS will be favored, thus promoting the stabilization of the (001) pseudo-steps.

**Figure 4.** Illustration of the arrangement of ions in the calcite (104) plane and Ca\(^{2+}\) density along the [001] step direction. View is normal to two monomolecular layers (bottom layer is transparent). A red dashed line indicates the calcium arrangement along a [001] step (for this given arbitrary step length four Ca\(^{2+}\) ions), with the [001] direction indicated by the red arrow. A green dashed line of equal length as the red line represents the calcium arrangement along the acute and obtuse steps on the (104) plane. The comparison of both lines shows that the calcium density along the [001] step is higher than those along the acute and obtuse steps on the (104) plane. The [001] step planes can be terminated by either a layer of calcium ions or a layer of carbonate ions. They contain a high density of 4.5 calcium ions nm\(^{-2}\) upon termination by a layer of calcium ions.[24]

Undoubtedly, the locally positively charged nature of an (001) pseudo-plane terminated by rows of calcium (Figure 4) will additionally contribute to its stabilization due to the electrostatic binding of PSS, as proposed in numerous other studies.[7a,9a,14,25] Molecular dynamics simulations of styrene sulfonate (SS) and PSS oligomer adsorption onto calcite surfaces[25] showed a preference for the (001) facet, where the sulfonate interaction takes place through direct and solvent-mediated binding, for which both the PSS-containing molecules showed an approximately perpendicular orientation toward
the surface. The solvent-mediated binding refers to the sulfonate group residing in the second layer of high water density which forms very strong hydrogen bonds with water molecules in the (strongly polarized) first and second solvation layers. In contrast, adsorption onto the less favorable (104) facet—where binding of the sulfonate to the surface is solvent-mediated by one or two layers of water molecules—can take place with the molecules oriented more parallel to the mineral surface. Because the structures of a [001] step and a {001} face are closely related, these two phenomena are likely to be related to one another and, as such, formation of the (001) pseudo-facets should reinforce PSS binding to the (001) plane and vice versa.

4. Conclusion

The findings presented here show that both the ammonium carbonate diffusion technique and the method of mixing CaCl₂ with NaHCO₃ to prepare CaCO₃ crystals in the presence of certain organic additives can eventually produce a morphology previously attributed to “mesocrystal” formation, in agreement with other recent work. Here, by comparing the outcomes from both methods with those from the in situ AFM observations, we have directly translated the resulting morphology from the nanoscale to the microscale and shown how it arises through completely classical growth mechanisms. Although we have demonstrated this using the specific case of PSS-modified calcite, the results may serve as a more general basis for extending classical growth mechanisms to the formation of other complex crystal morphologies. In addition, they reemphasize that proposals of nonclassical pathways based solely on observations of crystal morphology and surface roughness must be carefully considered.

5. Experimental Section

Detailed information on the following methods can be found in the Supporting Information: CaCO₃ benchtop diffusion experiments in the presence or in the absence of PSS, CaCO₃ overgrowth bench-top diffusion experiments, confocal Raman microscopy, atomic force microscopy overgrowth experiments on calcite, diffusion experiments in the AFM fluid cell, ion-selective electrode experiments, SEM, and cryo-TEM analysis.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.
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Conflict of Interest

The authors declare no conflict of interest.

Keywords

atomic force microscopy, calcium carbonate, crystal growth

References


Supplementary Information

A Mesocrystal-like Morphology Formed by Classical Polymer-Mediated Crystal Growth

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1. Materials and Methods

1.1. Reagents

All reagents were from Sigma Aldrich and were used as received: Calcium chloride dihydrate (powder, ACS reagent, ≥ 99%), poly(sodium 4-styrenesulfonate) (PSS, powder, average molecular weight ($M_w$) ~70,000 g mol$^{-1}$, degree of polymerization (DP) = 340), ammonium carbonate (powder, ACS reagent, ≥ 30% NH$_3$ basis), sodium chloride (powder, ACS reagent, ≥ 99%), NaOH (pellets, ACS reagent, ≥ 97%) and sodium bicarbonate (powder, ACS reagent, ≥ 99.7%). All aqueous solutions were prepared using Milli-Q water (18.2 MΩ, Millipore, 20 °C).

1.2. CaCO$_3$ bench-top diffusion experiments

1.2.1. In the presence of PSS

Bench-top crystallization experiments were carried out in a desiccator by utilizing the ammonium carbonate diffusion method as described in our previous work$^{[1]}$. A glass vial (28 mm diameter) was filled with 5 mL Ca-PSS solution, which was prepared by combining 0.05 g PSS powder with a 100 mL 1.25 mM CaCl$_2$ solution after vigorously stirring in a bottle. As a substrate, either a back-etched Si$_3$N$_4$/Si(100)/Si$_3$N$_4$ wafer (Si$_3$N$_4$ layer of 50–100 nm) or a cleaved mica substrate (both plasma-cleaned in air atmosphere for 1 minute) was used. This substrate was then placed at the bottom of the vial. Sealing the vial was performed using parafilm with three small holes punctured by a small needle. Two petri-dishes with each 0.5 g (NH$_4$)$_2$CO$_3$ were placed at the bottom of the desiccator and similarly covered with parafilm containing three punctured holes.

1.2.2. In the absence of PSS

Control experiments without the PSS were conducted according to the procedure described above, but instead using only 1.25 mM CaCl$_2$ as the mineralizing solution.
1.3. CaCO$_3$ overgrowth bench-top diffusion experiments

The crystals obtained by the control diffusion experiments on the Si$_3$N$_4$ wafer after 1 day of diffusion as described in Section 1.2.2 were quenched in ethanol. The wafer was dried by supplying a gentle N$_2$ stream and subsequently used again for overgrowth experiments, where the procedure in Section 1.2.1 was repeated in the desiccator with newly injected similar concentrations of growth solution (1.25 mM CaCl$_2$; 0.5 g L$^{-1}$ PSS).

1.4. Confocal Raman Microscopy

The CaCO$_3$ crystals grown on the Si/Si$_3$N$_4$ wafers in the unseeded bench-top and seeded overgrowth experiments were quenched in ethanol and analyzed using a LabRAM ARAMIS Raman microscope (Horiba Scientific). A 100× objective was used to image the crystals in the optical microscope, and a laser (wavelength 532 nm) was focused on the crystal of interest on the substrate using a D2 filter with a hole size of 300 μm. An acquisition time of one minute was used to obtain spectra with a resolution of 4 cm$^{-1}$ in the range of 100–2000 cm$^{-1}$.

1.5. Atomic Force Microscopy

A Digital Instruments Multimode Nanoscope IIIa (Veeco Metrology, Inc., Santa Barbara, CA) was used to perform in situ experiments in a sealed glass fluid cell. All images were acquired at room temperature and pressure utilizing a Si$_3$N$_4$ cantilever (Bruker, NP-S, spring constant 0.12 N m$^{-1}$) with a substrate and acquisition mode depending on the experiments described below. Image analysis was performed with Nanoscope Analysis v.1.40 software.
1.5.1. Overgrowth experiments on calcite

All images in the overgrowth experiments were performed using contact mode AFM. A freshly cleaved Iceland Spar single calcite crystal (Ward’s Scientific, Chihuahua, Mexico) with dimensions of approximately $10 \times 10 \times 3$ (l x b x h) mm was glued using a two component epoxy glue on top of a 15 mm diameter AFM metal specimen disk (Ted Pella, Redding, CA). An O-ring, which was inserted into the glass fluid cell, sealed the gap between the calcite face and the glass. The supersaturated growth solutions were made by the dissolution of reagents NaHCO$_3$ and CaCl$_2$, and NaCl into milli-Q water and adjusted by addition of 0.5 M NaOH to a final pH of 8.5, either in the presence or absence of PSS. The PSS-free solution contained concentrations of 2.2 mM CaCl$_2$, 4.4 mM NaHCO$_3$ and 37.1 mM NaCl, while the PSS-bearing solution contained 1.5 mM CaCl$_2$, 3 mM NaHCO$_3$, 41.1 mM NaCl and 0.1 g L$^{-1}$ PSS. The ionic strength of all solutions was set to ~0.05 M. The supersaturation $\sigma$ was defined as

$$\sigma = \ln \left( \frac{a(\text{Ca}^{2+}) \times a(\text{CO}_3^{2-})}{K_{sp}} \right)$$

where $a(\text{Ca}^{2+})$ and $a(\text{CO}_3^{2-})$ are the calcium and carbonate activity respectively, and $K_{sp}$ is the solubility constant. Here, the solubility constant for calcite $K_{sp, \text{calc}} = 10^{-8.48}$ M$^2$ (after ref. [2]) and $K_{sp, \text{ACC}} = 10^{-6.39}$ M$^2$ (after ref. [3]) at room temperature. Using Visual MINTEQ software[4], the values of $\sigma$ were determined with respect to calcite ($\sigma_{\text{calc}}$) and ACC ($\sigma_{\text{ACC}}$). In the case of the addition of PSS to the growth solution, due to Ca$^{2+}$ binding to the sulfonate groups[1], the actual Ca$^{2+}$ activities/concentrations were determined using a Ca$^{2+}$ ion selective electrode (Ca-ISE, see Section 1.6), and the calculated supersaturation was corrected accordingly. Reactant solutions were continuously pumped into the flow cell with a rate of 0.1 mL min$^{-1}$.

1.5.2. Diffusion experiments in the AFM fluid cell

The fluid cell was injected with growth solution (1.25 mM CaCl$_2$; 0.5 g L$^{-1}$ PSS), and the inlet and outlet of the liquid cell were connected with tubing containing a small amount of fresh (NH$_4$)$_2$CO$_3$ in the closed connected system. Imaging was performed on a freshly cleaved mica substrate. After a certain diffusion
time, the substrate with the obtained CaCO₃ crystals was quenched in ethanol and dried by supplying a gentle N₂ stream.

1.6. Ion Selective Electrode experiments

For Ca²⁺ concentration determinations, a computer-controlled automated titration system (Titrando 809, Metrohm, Switzerland) was used with a calcium ion selective electrode (Ca-ISE), pH reference electrode, and two Dosing units (Dosino 807, 2 mL glass cylinder). One Dosino contained a 10 mM NaOH solution to ensure a constant pH of 8.5.

1.6.1. Calibration of the ISE and pH electrode

By using a Nernstian approach, the Ca-ISE calibration was performed by the correlation of measured calcium potentials (U(Ca²⁺)) with known analytical concentrations. The pH meter was initially calibrated using pH 4.0, 7.0 and 9.0 Metrohm buffer solutions, while the Ca-ISE was calibrated by titrating in a 0.1 M CaCl₂ solution at a rate of 10 μL min⁻¹ into 25 mL milli-Q, which was stirred continuously at a constant rate.

1.6.2. Determination of free calcium concentration in the growth solutions

The potential of 25 mL growth solution either in the presence or in the absence of PSS under similar conditions (1.5 mM CaCl₂, 3 mM NaHCO₃, 41.1 Mm NaCl, including or excluding 0.1 g L⁻¹ PSS) was measured at pH 8.5 after the aforementioned calibration procedure. The Davies equation was used to convert the measured Ca²⁺ activities into concentrations by determination of the activity coefficients, which were additionally verified by Visual MINTEQ.

1.7. Scanning Electron Microscopy

Imaging was performed using a Zeiss ULTRATM 55 field emission Scanning Electron Microscope (SEM) with an accelerating voltage of 3 kV. Crystals obtained from bench-top (overgrowth) diffusion experiments
on mica or Si₃N₄ were quenched in ethanol and subsequently dried by supplying a gentle N₂ flow and transferred directly to a metal stub for analysis without further treatments.

1.8. Cryo-TEM Analysis

Vitrification Procedure - Sample vitrification was performed by using an automated vitrification robot (FEI Vitrobot™ Mark III). Sample supports (type R2/2 Quantifoil Jena) were purchased from Quantifoil Micro Tools GmbH, and contained a carbon support film on a copper grid. Prior to use, the TEM grids were glow discharged by a Cressington 208 carbon coater to render them hydrophilic. Cryo-samples were prepared from a 3 µL droplet of sample solution placed on the grid inside the Vitrobot™ chamber at 100% relative humidity and a temperature of 20 °C, after which it was blotted to remove excess solution (blotting time 3 s, blot offset –3) and subsequently plunged into liquid ethane for vitrification.

Imaging of the vitrified samples - A Titan Krios™ (FEI) equipped with a field emission gun (FEG) and operating at 300 kV was used for high resolution imaging. Image recording was performed using a GIF 2002 Gatan energy filter connected to a 2K x 2K pixel MultiScan™ CCD camera.
2. Supporting Data

2.1. Bulk diffusion experiments

Figure S1. Growth of calcite-PSS crystals on the Si$_3$N$_4$ substrate. a) Schematic of the CaCO$_3$ bench-top diffusion setup, where the inset shows the orientation of the Si/Si$_3$N$_4$ wafer (90° with respect to the countertop), on top of which calcite-PSS crystals nucleate and grow. This rules out sedimentation of formed crystals onto the substrate. b) SEM image of a typical calcite-PSS crystal after 1 day diffusion (scale bar 5 μm), with c) a zoom-in of the typical roughened (001) facet (scale bar 5 μm).

The typical calcite-PSS crystal morphology was observed for the majority of the calcite crystals present on the substrate, however, occasionally a more traditional rhombohedral morphology of calcite crystals was observed.
The overgrowth diffusion experiments were started by using single crystal calcite seeds (Figure S2b, Materials and Methods) and after a day of diffusion a typical calcite-PSS morphology was formed (Figure S2c). However, simultaneously a typical island-growth pattern formed occupying a large portion on the (104) facets (Figure S2d), indicating the occurrence of surface nucleation of calcite in contrast to the case from the regular unseeded bulk diffusion experiments. Occasionally, we observe spherical vaterite particles on the surface of the calcite-PSS crystals (see e.g. Figure S2d), likely due to sample preparation artifacts (e.g. quenching of the solution).

Figure S2. Size and morphology of calcite-PSS crystals in diffusion bulk experiments compared to overgrowth diffusion experiments on calcite seeds. a) Size of the calcite-PSS crystals (expressed by the maximum Feret diameter: \(d_{\text{Fe,max}}\)) vs. diffusion time. The blue data points correspond to measurements from the diffusion bulk experiments (1.25 mM CaCl\(_2\); 0.5 g L\(^{-1}\) PSS as mineralizing solution) shown in Figure S1. The red data points represent measurements from the overgrowth experiments. A logistic function is used to fit each set of data points. The error bars represent the standard deviation. The red data points labeled as b-e, indicate crystal sizes at a specific diffusion time, which correspond to the b-e) SEM images on the right. b) is a typical single crystal calcite seed as in Figure 1a (scale bar 5 \(\mu\)m). (c-d) are examples of overgrown seeds after two days of diffusion. The inset in d) shows a zoom-in of the white square (scale bars 10 \(\mu\)m). e) shows a typical calcite-PSS crystal after five days of total diffusion (scale bar 10 \(\mu\)m).

Teng et al.\(^{[5]}\) showed that when the supersaturation (\(\sigma\)) increased to reach a critical value, steps began to be generated by what appeared to be a homogeneous (or two-dimensional) surface nucleation mechanism. At intermediate \(\sigma\), growth originated from both hillocks and by two-dimensional surface nucleation, where
the latter formed predominantly within flat areas on the surface rather than on terraces of spiral hillocks. Thus, at higher $\sigma$ the nucleation rate and density of 2D nuclei increased, showing that the surface nucleation mechanism was increasingly dominant. Although we used the same initial calcium concentration (1.25 mM) in both unseeded and seeded experiments, we observed a higher amount of vaterite in the seeded case (Figure S5e). While in the unseeded case calcite formed through dissolution and reprecipitation of vaterite, in the seeded case this calcite was already present. Consequently, in the seeded experiments more vaterite formed and was available to dissolve and reprecipitate as calcite, resulting in the surface nucleation mechanism observed on the seeds at higher $\sigma$.

However, after 5 days of total diffusion, the island growth appears to have been vanished (Figure S2e), indicating this was present only in a certain regime of the reaction process. The presence of 2D surface nucleation would explain the more roughened appearance of the (104) facets observed in the overgrowth diffusion calcite-PSS crystals compared to those from the regular unseeded diffusion experiments. Nevertheless, the similarity in the morphology of the end product is evident.
**Figure S3.** a,b) Etching experiments of calcite-PSS crystals observed in SEM images and c) Raman spectra of calcite-PSS crystals selected with optical microscopy in diffusion bulk experiments vs. overgrowth diffusion experiments. a) A typical calcite-PSS crystal obtained after 1 day diffusion. b) A typical example of a calcite-PSS crystal after 1 day of diffusion etched for 2 hours in DI water. The yellow arrows indicate typical observed etch pits at the (104) facets. Scale bars in a,b) are 5 μm. Two Raman spectra are given in c) where the red curve represents the spectrum of a calcite-PSS crystal observed in overgrowth diffusion experiments and the blue curve that of a calcite-PSS crystal in regular diffusion experiments.
2.2. Formation of vaterite on the substrate

Figure S4. Presence of vaterite after longer diffusion times on mica and Si$_3$N$_4$. a) SEM image of CaCO$_3$ crystals after 5 days of diffusion on Si$_3$N$_4$, which shows – in addition to three clear calcite-PSS crystals – numerous vaterite crystals (examples are indicated by yellow arrows), distinguishable by their rounded morphology (scale bar 20 μm). b) SEM image of grown CaCO$_3$ crystals after 5 days of diffusion on mica; examples of vaterite crystals are indicated by yellow arrows. The inset shows a clearer zoom-in of a calcite-PSS crystal with similar morphological features to those in a). Scale bars are 20 μm. c) Raman spectrum taken of a typical rounded crystal in the inset (scale bar 10 μm) showing vaterite peaks (in red). The peaks at 520 and 970 cm$^{-1}$ (in black) are present in the background, originating from the Si$_3$N$_4$ wafer, of which the Raman spectrum is shown in d). The peak at 520 cm$^{-1}$ belongs to Si and the band at 970 cm$^{-1}$ to the Si-OH stretch (ref. [6]).
Figure S5. Nucleation of calcite-PSS crystals by dissolution-reprecipitation of vaterite. a-c) SEM images of the reaction progress on mica in the AFM fluid cell after different diffusion times: a) vaterite crystals formed after ~2h of diffusion (scale bar 10 μm), b) vaterite formed after ~4h of diffusion (scale bar 10 μm) with an observable calcite-PSS crystal on the surface, indicated by the yellow arrow and enlarged in the inset (scale bar 1 μm) and c) diffusion of ~1 day led to more calcite-PSS crystals indicated by the yellow arrows (scale bar 10 μm). d-e) show crystals obtained by bulk diffusion experiments after 5 days of diffusion (scale bars 50 μm), indicating observable depletion zones by the yellow arrows. d) images from the regular unseeded diffusion experiments, with the depletion zones surrounding two calcite-PSS crystals. e) shows the crystals obtained by the overgrowth diffusion experiments, where the amount of vaterite that has nucleated is substantial and the depletion zones surrounding the calcite-PSS crystals are apparent. The inset shows a zoom-in of such a depletion layer (scale bar 10 μm).

The solubility of the calcium carbonate polymorphs is the key factor controlling growth of calcite during the stage when vaterite transforms to calcite. When there is vaterite present, the composition of the solution will be controlled by the solubility of this phase, as the solubility of vaterite is larger than that of calcite ($K_{sp, vat} = 10^{-7.913}$ vs. $K_{sp, cal} = 10^{-8.480}$, respectively). While the transformation of vaterite to calcite is slow (rate-determining factor), dissolution of vaterite will only occur at an ionic activity product corresponding to or below the solubility product of vaterite. Once the vaterite fully dissolves, the solution will remain supersaturated with respect to calcite driving the precipitation of this phase.
The ion activity product should be lower than approximately $10^{-7.913} = 1.2 \times 10^{-8}$ M$^2$ for vaterite to dissolve in the benchtop experiments, while in our *in situ* AFM overgrowth experiments on the calcite seed, the ion activity product of the prepared solution was slightly higher at $1.8 \times 10^{-8}$. However, the actual supersaturation level within the fluid cell where calcite grew was smaller than this value, and could be even less than the solubility of vaterite. This is because the solution flow rate we used (0.1 ml min$^{-1}$) was not sufficiently fast to replenish calcium and carbonate ions into the fluid cell at a rate equal to their depletion rate within the fluid cell where the seed calcite grew. Flow rates higher than 0.1 ml min$^{-1}$ did not allow us to monitor the overgrowth process accurately in time, because: 1) at these rates growth was too fast to follow the resulting morphological change; 2) at higher flow rates, larger concentrations of PSS enter the AFM fluid cell, which can significantly scatter the laser beam into the photodiode detector, resulting in reduced image quality. Therefore, the lower flow rate allowed us to observe growth with a suitable image quality. However, based on our observations at the high flow rates, as well as in the quiescent conditions of benchtop experiments, the key factor in producing the resulting morphological change – which was shown to transfer from the nanoscale to the microscale – is the interaction of the growth steps with PSS and is not significantly affected by these differences in supersaturation.

2.3. Role of ACC in calcite-PSS crystal formation: benchtop vs. *in situ* experiments

We assign the relatively low interaction of PSS with free calcium ions as determined in Figure S6a to 1) the relatively low amount of polymer that is present in the solution and 2) the presence of a substantial amount of CO$_3^{2-}$ and HCO$_3^-$, which can interfere with Ca$^{2+}$ binding to the sulfonate group of PSS.

When vaterite is dissolving in the benchtop experiments, it is redundant to investigate the presence of ACC with the reported ACC solubility product of Brečević *et al.*[^3]. However, in our *in situ* AFM overgrowth experiments we did not observe vaterite formation ($\sigma$ with respect to vaterite using this solubility product is lower than 0.39, as discussed above). Therefore, it is important to exclude growth by amorphous particle addition in the *in situ* experiments. As the main manuscript text explains (see section: Role of ACC in calcite-PSS crystal formation), we do not find evidence of growth through addition of ACC particles.
If we take the lowest reported value for the ACC solubility product by Gebauer et al. ($K_{sp,\text{ACC}} = 10^{-7.5}$, see ref. [7]) we obtain a $\sigma$ with respect to ACC of -0.55, i.e. the solution is still undersaturated with respect to ACC. We therefore conclude that the observed growth in our experiments did neither occur by oriented attachment of crystalline particles, nor by amorphous particle addition.

![Graph](image1.png)

**Figure S6.** Investigation of ACC particle formation in the growth solution. a) Free calcium concentrations ($c(\text{Ca}^{2+})_{\text{free}}$) as determined by a Ca-ISE as a function of time in the overgrowth solution: without PSS (red line), containing the PSS (black line), and difference between both (blue line) as a result of $\text{Ca}^{2+}$ binding by the PSS. Error bars are corresponding standard deviations. PSS binds $0.12 \pm 0.05 \text{ mM Ca}^{2+}$ (mean ± s.d. of the distribution) of the total present calcium in solution (or in percentage $8.0 \pm 3.3\%$). b) cryo-TEM image of the overgrowth solution, with PSS particles indicated by yellow arrows. The inset shows a zoom-in of the image in b) with two additional particles indicated by the yellow arrows. Scale bars are 10 nm. c) DLS volume distribution. The low count rate, as indicated, is in agreement with the low concentration of globules as observed by cryo-TEM. In both DLS and cryo-TEM, no evidence was observed for the presence of larger ACC particles.
2.4. Comparison of calcite-PSS crystals with cleaved single calcite crystals

Figure S7. a) Grown calcite-PSS crystal obtained by bulk diffusion experiments after 5 days (scale bar 10 μm). b) Simulated calcite single crystal (SHAPE V7.3) with cleaved (001) and (018) family planes modified and oriented to match the crystal in a). c) Grown calcite-PSS crystal obtained by overgrowth diffusion experiments on single crystal calcite seeds after 4 days (scale bar 10 μm). d) Simulated calcite single crystal with cleaved (001) and (018) family planes modified and oriented to match the crystal in c).
2.5. Height changes in calcite step direction before and after PSS modification

![Figure S8](image.png)

**Figure S8.** Modification of obtuse and acute steps by PSS as imaged by in situ AFM. a) Height profile across the obtuse steps without PSS (solid black line) and with PSS (solid green line) and b) height profile across the acute steps without PSS (dashed black line) and with PSS (dashed green line), as measured by corresponding line-scans in the AFM height images of c) and d), respectively. Here, c) is the calcite growth hillock without PSS and d) with PSS added in the growth solution. Scale bars in c) and d) are 500 nm.

As shown in **Figure S8**, the acute steps seem to be much more roughened than the obtuse steps, suggesting that the PSS preferentially bound to the acute over obtuse steps.
3. Supporting references


