On the Adsorption of Aspartate Derivatives to Calcite Surfaces in Aqueous Environment

Robert Stepic, Lara Jurković, Ksenia Klementyeva, Marko Ukrainczyk, Matija Gredičak, David Matthew Smith, Damir Kralj, Ana-Sunčana Smith

Submitted date: 09/01/2020 • Posted date: 09/01/2020
Licence: CC BY-NC-ND 4.0

Citation information: Stepic, Robert; Jurković, Lara; Klementyeva, Ksenia; Ukrainczyk, Marko; Gredičak, Matija; Smith, David Matthew; et al. (2020): On the Adsorption of Aspartate Derivatives to Calcite Surfaces in Aqueous Environment. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.11558430.v1

In many living organisms, biomolecules interact favorably with various surfaces of calcium carbonate. In this work, we have considered the interactions of aspartate (Asp) derivatives, as models of complex biomolecules, with calcite. Using kinetic growth experiments, we have investigated the inhibition of calcite growth by Asp, Asp2 and Asp3. This entailed the determination of a step-pinning growth regime as well as the evaluation of the adsorption constants and binding free energies for the three species to calcite crystals. These latter values are compared to free energy profiles obtained from fully atomistic molecular dynamics simulations. When using a flat (104) calcite surface in the models, the measured trend of binding energies is poorly reproduced. However, a more realistic model comprised of a surface with an island containing edges and corners, yields binding energies that compare very well with experiments. Surprisingly, we find that most binding modes involve the positively charged, ammonium group. Moreover, while attachment of the negatively charged carboxylate groups is also frequently observed, it is always balanced by the aqueous solvation of an equal or greater number of carboxylates. These effects are observed on all calcite features including edges and corners, the latter being associated with dominant affinities to Asp derivatives. As these features are also precisely the active sites for crystal growth, the experimental and theoretical results point strongly to a growth inhibition mechanism whereby these sites become blocked, preventing further attachment of dissolved ions and halting further growth.

File list (2)

| File                  | Size          | Link
|-----------------------|---------------|-------------------------------|
| ASP-230919-Manuscript.pdf | 773.33 KiB   | [view on ChemRxiv](#) | [download file](#) |
| ASP-230919-SI.pdf    | 1.78 MiB     | [view on ChemRxiv](#) | [download file](#) |
On the Adsorption of Aspartate Derivatives to Calcite Surfaces in Aqueous Environment

Robert Stepić,†,§ Lara Jurković,†§ Ksenia Klementyeva,† Marko Ukrainczyk,‡ Matija Gredičak,¶
David M. Smith,†,* Damir Kralj,‡,* and Ana–Sunčana Smith†,*

† Group for Computational Life Sciences, Division of Physical Chemistry, Ruđer Bošković Institute (RBI), Bijenička cesta 54, 10000 Zagreb, Croatia
‡ PULS Group, Institute for Theoretical Physics and Interdisciplinary Center for Nanostructured Films, FAU Erlangen–Nürnberg, Cauerstrasse 3, 91058 Erlangen, Germany
§ Laboratory for Precipitation Processes, Division of Material Chemistry, RBI, Bijenička cesta 54, 10000 Zagreb, Croatia
¶ Laboratory for Biomimetic Chemistry, Division of Organic Chemistry and Biochemistry, RBI, Bijenička cesta 54, 10000 Zagreb, Croatia

Supporting Information

ABSTRACT: In many living organisms, biomolecules interact favorably with various surfaces of calcium carbonate. In this work, we have considered the interactions of aspartate (Asp) derivatives, as models of complex biomolecules, with calcite. Using kinetic growth experiments, we have investigated the inhibition of calcite growth by Asp, Asp; and Asp. This entailed the determination of a step-pinning growth regime as well as the evaluation of the adsorption constants and binding free energies for the three species to calcite crystals. These latter values are compared to free energy profiles obtained from fully atomistic molecular dynamics simulations. When using a flat (104) calcite surface in the models, the measured trend of binding energies is poorly reproduced. However, a more realistic model comprised of a surface with an island containing edges and corners, yields binding energies that compare very well with experiments. Surprisingly, we find that most binding modes involve the positively charged, ammonium group. Moreover, while attachment of the negatively charged carboxylate groups is also frequently observed, it is always balanced by the aqueous solvation of an equal or greater number of carboxylates. These effects are observed on all calcite features including edges and corners, the latter being associated with dominant affinities to Asp derivatives. As these features are also precisely the active sites for crystal growth, the experimental and theoretical results point strongly to a growth inhibition mechanism whereby these sites become blocked, preventing further attachment of dissolved ions and halting further growth.

Minerals with exceptional mechanical properties are known to form when an inorganic crystal is grown in a medium that contains dissolved organic molecules. This process, known as biominalization, has garnered a lot of attention in recent years due to its relevance in functional material design. The current hypothesis states that this phenomenon is predominantly driven by favorable electrostatic interactions between the inorganic ions forming the crystal lattice and biological matter in the form of negatively charged polyelectrolytes, mainly peptides, proteins, and polysaccharides. Consequently, aspartic acid (Asp), which has a negatively charged side chain at biological pH, is found to be one of the most prominent constituents of bioactive peptides and proteins, while the most studied minerals in this context are the naturally abundant carbonates, and in particular, calcite. Calcite grown in such an environment shows remarkable tensile strength and complete biocompatibility making it a major candidate for many potential applications, the most prominent being in oil reservoirs, CO₂ storage, and especially drug delivery systems. Despite this enormous potential, predictive understanding of the growth is still missing.

Inorganic calcite (CaCO₃) appears in various morphologies, of which the rhombohedrons bounded by the most stable calcite surfaces, the (104) faces, are the most frequent. In contrast, many different shapes are exhibited in crystals grown in biological matrices as a result of the competition of constituent ions with organic molecules for the surface binding sites on the interfaces. Consequently, the kinetic analyses of additive-inhibited crystal growth is one successful avenue of investigation, in particular, allowing the extraction of adsorption constants of a biomolecule to an inorganic surface, and allowing for the inference of the growth mode. Specifically, if the additives bind strongly to active sites on the surfaces, they may block the sites and thus impair the crystal growth (step pinning). Typically, this scenario is associated with a critical supersaturation of aqueous ions, at which growth can no longer occur (“dead zone”). An alternative scenario, known as kink blocking, is typical for impurities that adsorb weakly and briefly to the kinks at growing steps. As this situation leads only to an effective reduction in propagation and the kinetic growth coefficient, no dead zone

Scheme 1. Predominant chemical structures of Asp, Asp; and Asp at nearly neutral pH.
should be observed.\textsuperscript{25} If, on the other hand, the additives are incorporated into the growing crystal to a significant extent, they may distort it and increase the internal free energy.\textsuperscript{26} This can lead to a lower effective supersaturation and a lower growth rate at a given concentration of constituent ions.\textsuperscript{27}

The free energy of binding is also accessible by molecular dynamics (MD) simulations,\textsuperscript{28,29,30,31} which, in addition, provide full atomic detail of the systems. However, because of the large number of available approaches and the sparsity of calibration against experiments,\textsuperscript{30,32} an obvious general methodology for modelling interactions on an arbitrary calcite interface is subject to several important challenges.\textsuperscript{33,34,35}

The first challenge is to model the interactions between the solid and the liquid phase.\textsuperscript{28,29,31} Motivated by the method proposed for zeolites,\textsuperscript{36} an attractive approach based on the AMBER\textsuperscript{37} force field was recently developed.\textsuperscript{38} However, the thereby-used\textsuperscript{39} and other\textsuperscript{40,41,30,42} models for the calcite are likely to be inferior for aqueous applications than a newer parametrization, which more accurately captures the thermodynamic properties of the water-calcite interface.\textsuperscript{41} Nevertheless, the combination of the most promising models for the organic\textsuperscript{45} and inorganic\textsuperscript{41} phases has not yet been tested. The second challenge is the geometry of the calcite. Even though the (104) surface is the most abundant in the equilibrium, a realistic crystal model should account for different defects. Besides affecting the local water structure,\textsuperscript{43,44} these features have different affinities for the organic additive, as shown recently for aspartic acid that demonstrated a preference to bind to acute edges and calcium corners.\textsuperscript{45} These latest results, however, were not calibrated against experiment, which is the third challenge in the simulation field. With reported binding energies of Asp to various calcite surfaces ranging from\textsuperscript{30} 2 kJ mol\textsuperscript{-1} to\textsuperscript{36} 400 kJ mol\textsuperscript{-1}, and with the reliability only addressed for the interactions of selected monomeric species with calcite,\textsuperscript{30,32} this aspect is of paramount importance. In particular, the significantly more complex challenge involving calibrated modeling of the binding of a polymeric series to calcite, has not yet been attempted.

In this work, we overcome these simulation challenges and determine the free energies of binding of an array of aspartate-based derivatives (Scheme 1), using a more realistic calcite surface, and advanced sampling techniques. By performing kinetic growth experiments, we are able to determine the growth regimes and generate benchmark values for the free energies of binding. Comparison of the modelling results with this data allows us to offer, with a high level of confidence, a systematic overview of the binding preferences of these biologically active building blocks to a complex calcite interface.

**Experimental Growth Inhibition.** Analytical grade L-Asp and (1-Asp); were used while the (L-Asp) derivative was synthesized automatically from C- to N-terminus by the solid-phase Fmoc method (see supporting information: Section S1). Calcite seeds were prepared by the semi-continuous carbonation method (see Section S2).\textsuperscript{47} The initial solution for crystal growth was composed of a mixture of calcium and carbonate ions, an Asp derivative and the calcite crystal seed. The growth experiments were performed in a thermostated double-walled glass vessel (see Section S3). The resulting change in pH, measured with a combined glass-calomel electrode, is induced by the incorporation of dissolved constituent ions (calcium and carbonate) into the crystal lattice, which caused changes in ionic equilibrium of carbonate ions to the growing crystal. Typical progress curves of calcite growth, pH versus time, obtained in the control system (black line), as well as in the systems containing different concentrations of Asp; are shown in Figure 1. It is evident that the calcite growth, indicated as a pH drop, started immediately after the addition of the seed in the systems (t=0). Relative to the control system, the crystal growth in the biomolecule-containing systems is progressively impeded by the increase in concentration of Asp. This is seen as the lowering of the slope of the respective curves. In addition, the growth in the presence of biomolecular additives apparently terminates at a specific pH (dead zone), which is higher for higher concentrations of the additive. This confirms that the added biomolecule binds relatively strongly to the available binding sites, impeding incorporation of calcium and carbonate ions and fully halting any further growth (step pinning). Additional data for the pH variation with different amounts of other additives (Asp and Asp), where we can observe a similar effect on growth, is given in Figure S1.

The solution composition at any moment of the process was calculated by using the measured pH and the known initial total concentrations of the reactants (Section S3). The detailed calculation procedure, which takes into account the respective protolytic equilibria and equilibrium constants, as well as the charge and mass balance equations, is described in previous work.\textsuperscript{47,48} The crystal growth rate, R, was then calculated by numerical differentiation of the dissolved calcium concentration with respect to time and normalizing the result by the total surface area. The total surface area of the calcite seed was estimated by the multiple Brunauer-Emmet-Teller method,\textsuperscript{48} and the increase of the area during growth was taken into account and calculated from the total calcium carbonate precipitate data. In terms of the supersaturation, the rate law can be described as $R = k_1(S - 1)\ln S$ (see Figure S2), as could be expected for the low-supersaturated systems.\textsuperscript{27}

The mode and extent of the interactions of the biomolecules with the crystal surface can be quantitatively estimated by the means of Kubota and Mullin’s mathematical model.\textsuperscript{25} This approach uses the inhibited growth rate $R$ relative to the growth rate in the absence of any additives, $R_0$, as the response variable and correlates it with the biomolecular coverage of the face active sites $\theta_{03}$, and their effectiveness $\alpha$:

$$\frac{R}{R_0} = 1 - \alpha \theta_{03}$$

(1)
In this model, the effectiveness factor is assumed to be a stereochemical contribution of the respective dissolved organic molecules to the growth reduction. It is tied to the size, shape or orientation of the interacting biomolecules with respect to the active surface sites.

In order to relate the concentration of the organic molecules in solution (cₐ) and the growth rate reduction, the Langmuir adsorption model and isotherm are applied:

\[ \frac{R}{R_0} = 1 - \alpha [K_{ad} c_a/(1 + K_{ad} c_a)] \]  

In this expression, \( K_{ad} \) is the Langmuir adsorption constant for the process of biomolecule binding to the calcite surface, \( R \) is the growth rate in the system with some concentration of biomolecules and \( R_0 \) is the growth rate in the control system at complete surface coverage. When \( \alpha > 1 \), the growth rate approaches zero even at incomplete surface coverage, while at \( \alpha < 1 \) the growth is not completely reduced, even at complete coverage of active sites for adsorption.

Figure 2 shows typical plots of the relative growth rate reduction (\( R/R_0 \)) for calcite seeds at different relative supersaturations as a function of different concentrations (cₐ) of Asp, Asp₂ and Asp₃, for selected supersaturations. The lines drawn through the experimental points are obtained by fitting the respective set of data with the function given by Eq. 2. We used a non-linear fitting procedure where two parameters were allowed to vary (\( K_{ad} \) and \( \alpha \)), and the concentration of the additive was used as the independent variable. Values of the adsorption constants do not depend on supersaturation. Thus, we have three independent measurements for each biomolecule. By taking the average over three independent fits, we obtained the following values of adsorption constants: \( K_{ad} = 1.0 \pm 0.1 \text{ dm}^3 \text{ mol}^{-1} \) (Asp); \( K_{ad} = 58.5 \pm 8.5 \text{ dm}^3 \text{ mol}^{-1} \) (Asp₂); \( K_{ad} = 397.2 \pm 19.7 \text{ dm}^3 \text{ mol}^{-1} \) (Asp₃). These values can be converted into the associated Gibb’s free energies of binding -17.1 ± 0.2 kJ mol⁻¹ (Asp), -27.2 ± 0.3 kJ mol⁻¹ (Asp₂), -32.0 ± 0.1 kJ mol⁻¹ (Asp₃), as shown in Table 1. The energy of binding of aspartate compares well with the value of -21 kJ mol⁻¹ obtained previously by the means of fitting Langmuir, Langmuir-Freundlich, and Flory-Huggins isotherms to the fractional growth inhibition.

The experimental results we report here indicate that the energy of binding increases as we add more units of aspartate to the peptide. However, it does not do so linearly but rather in an asymptotic fashion. This also agrees with the hypothesis proposed in the literature.

In addition to the adsorption constants, we determined the relation between the biomolecular effectiveness factor \( \alpha \) and the supersaturation (Figure 2, insets). In all cases, we obtain 1 < \( \alpha < 3 \), which is consistent with the step-pinning mechanism. We also observe that \( \alpha \) is the highest for Asp, followed by Asp₂, whereas Asp₃ is the least effective of the three at comparable supersaturation values. Additional data for \( \alpha \), over a wider range of supersaturations, is presented in Figure S3.

### Theoretical Binding Energies

We begin by considering the binding of the Asp-derivatives (Asp, Asp₂, Asp₃) to a flat (104) surface, (Section S5) primarily using the GROMACS 4.5.5 simulation package (see Section S6 for other software employed). After an equilibration protocol, we performed umbrella-sampling simulations (147 ns/peptide, see Section S7) to calculate the potential of mean force (PMF). The combination of the multiple umbrella sampling runs is performed by weighted histogram analysis method (WHAM). As a reaction coordinate, we use the vertical distance of the center of mass of the Asp-derivative from the first layer of the calcite crystal (zCOM). The position of the first layer is defined as the average position of interfacial calcium ions.

Parameters for the zwiterionic Asp-derivatives are extracted from the AMBER force field and combined with TIP3P water. Given that the thermodynamics of the interfacial water is an important determinant of the derivative binding to calcite, the crystal is modeled using the force field of Raiteri et al. The classic combination rules for organic-inorganic interactions are avoided as much as possible, because of the significant disparity in the charge magnitudes in the two subsystems, following the recommendation of Freeman et al. Accordingly, the cross-terms involving calcium

|                | Experiment | Simulations (104) | Simulations (island) |
|----------------|------------|-------------------|----------------------|
| Asp            | -17.1 ± 0.2 | -30 ± 1           | -13 ± 1              |
| Asp₂           | -27.2 ± 0.3 | -19 ± 1           | -20 ± 1              |
| Asp₃           | -32.0 ± 0.1 | -15 ± 1           | -23 ± 1              |

*Comparative to the value of 21 kJ mol⁻¹ in Ref. 23 (b) Value of -46 kJ mol⁻¹ is obtained using parametrization from Refs 30 and 36.
are obtained using the Schroeder\textsuperscript{16} method, the interactions with the oxygen were evaluated using Lorentz-Berthelot combination rules, while the interactions with carbon were taken from the AMBER\textsuperscript{37} force field (further details on the force field are given in Section S8). In combination with a simple model for calcite,\textsuperscript{19} this approach yielded very accurate results for different organic functional groups, when compared to the ab initio data.\textsuperscript{16}

Combining these cross-terms with the Raiteri calcite force field, and calculating the PMF for Asp binding to the neutral (104) surface (Figure S6) provides a very high binding energy (-46 kJ mol\(^{-1}\)), well outside of the experimental range. After a careful analysis, we find the source of this over-binding in the dispersion interactions of crystal oxygen with aspartate atoms, which are therefore replaced by the AMBER force field\textsuperscript{39,41} parameters, effectively eliminating the need to use any combination rules for inorganic-organic cross terms (see Tables S1-S3). The calculation of the PMF (Figure S6) with this modification yields a 16 kJ mol\(^{-1}\) reduction of the binding free energy, which now adopts a value of -30 kJ mol\(^{-1}\), significantly closer to the experimental measurements. At the same time, the crystal-water interface remains intact, consistent with the thermodynamics of solvation of calcium and carbonate ions.

We repeat the analogous simulation procedure and constructed two additional free energy profiles for the binding of Asp\(_2\) and Asp\(_3\) to the (104) surface of calcite (Figure S7). As shown in Table 1, the free energy of binding of Asp\(_2\) amounts to -19 kJ mol\(^{-1}\) and for Asp\(_3\) it is -15 kJ mol\(^{-1}\). Not only do the individual binding energies deviate from the experiment, but also the experimental trend within the series is not recovered. This indicates an issue beyond the level of the force field. For this reason, we introduce a calcite surface with an island possessing six distinct features (see Section S5).\textsuperscript{14} acute (AE) and obtuse (OE) edges, acute (ACC) and obtuse carbonate (OCC) corners, a calcium corner (CaC), and a central surface exposing a (104) plane (MI).

To evaluate the binding of Asp, Asp\(_2\), and Asp\(_3\) to such a complex surface, we first equilibrate each Asp-derivative on each of the six features and produce six 147 ns sampling runs (total of 882 ns/Asp-derivative), which are consequently combined using WHAM. The corresponding average profiles for Asp, Asp\(_2\) and Asp\(_3\) (Figure 3) provide the free energies of binding of -13 kJ mol\(^{-1}\), -20 kJ mol\(^{-1}\) and -23 kJ mol\(^{-1}\), respectively (Table 1). Despite being slightly, yet systematically too low, these binding energies are in an excellent agreement with experimental data, especially for such a challenging series.

**Analysis of dominant interactions.** With the reliability of the computational model established, we turn to investigating the binding modes of the Asp-derivatives to the calcite surfaces.

We first assess the frequency of the interactions with the various island features, using a self-developed analysis tool (Section S10). This results in a set of normalized histograms (Figure 4), which

![Figure 3](image-url) **Figure 3.** Interactions of Asp derivatives with the calcite surface containing an island (a) Potentials of mean force for binding of Asp, Asp\(_2\) and Asp\(_3\). Structures representing common binding modes for (b) Asp, (c) Asp\(_2\), and (d) Asp\(_3\).

![Figure 4](image-url) **Figure 4.** Probability of a biomolecule (Asp, Asp\(_2\), Asp\(_3\)) to be bound to a certain feature of the island: acute carbonate corner (ACC), acute edge (AE), calcium corner (CaC), central surface exposing a (104) plane (MI), obtuse carbonate corner (OCC), obtuse edge (OE), or unbound (UB).
show that Asp and Asp₃ have a preference for binding to the acute edge and the calcium corners, similar to that reported for Asp previously. This preference seems to diminish for Asp₃, which is in line with investigations of the inhibited growth of a series of Asp derivatives with Atomic Force Microscopy. Further inspection of Figure 4 shows that all of the Asp-derivatives, however, tend to be drawn from the middle to the edges and corners of the island. Given that these features are precisely the active sites during crystal growth; this finding explains the inhibition of growth upon the addition of Asp-derivatives (Figure 1 and S1). Furthermore, the relatively large binding affinities are in agreement with the appearance of the experimentally obtained dead zone and the complete blocking of the growth at sufficient additive concentration.

The overall binding free energies (and the associated binding modes) are, however, the result of interactions between not only the additive and the features of surface but they also contain large contributions from the (de)solvation of the Asp derivatives and the ions on the calcite surface, in a unique way over each feature of the surface. However, several common aspects, which seem to dominate the interactions of the Asp derivatives, can be extracted. For example, the Asp monomer, in its lowest-energy minimum on the island (Figure 3) and for the most prominent bonding modes identified for each of the six surface features (Figure S9, Table S4), displays a persistent interaction between the positively charged ammonium group and the solid phase. The majority of these modes also exhibit binding through the C-terminal carboxylate, whereas the sidechain carboxylate remains solvated in all cases. Interestingly, analogous features are associated with the dominant minimum for Asp on the (104) surface (Figure S6).

For Asp on the island (Figures 3 and S10), the situation is very similar to that for Asp. Specifically, the ammonium group establishes a persistent (yet slightly more distant, Table S5) contact with the calcite, whereas at most one of three carboxylate groups is also bound to the surface. This leaves at least two carboxylate groups in solution, explaining the larger values of zCOM associated with the minimum in the overall PMF (Figure 3). On the flat (104) surface both Asp₂ and but also Asp₃ show the ammonium group always bound to the surface together with at most one carboxylate group also bound (Figure S8). The remaining carboxylate groups continue to exhibit a strong preference for being solvated. This preference appears to be responsible for the positions of the minima being shifted to higher values of zCOM and, indeed, the decreasing binding energies as the number of Asp units is increased (Table 1).

Like Asp, Asp₃ on the island (Figures 3 and S11) also exhibits a dominance of ammonium-mediated binding (including close contacts, Table S6). However, in this case, at most two carboxylates are found in direct simultaneous contact with the calcite, while at least two appear in the aqueous medium. More often than not, the bound carboxylates originate from one of the side chains so that, on average, the COM of Asp₃ is closer to the surface than that of Asp. Precisely this feature should be seen in the context of the efficiency for the growth inhibition of Asp, relative to Asp (larger values of α for comparable supersaturation), which is obviously a stereochemical contribution, not just a consequence of binding affinity. This effect is likely to be associated with the fact that Asp₃ (as well as Asp) can access the crystal edges more closely than Asp, as discussed above, and is thus able to inhibit the crystal growth more effectively. Importantly, such a conclusion is only extractable from the island model, as the derivative accessibility to the flat (104) surface offers the opposite trend.

Summary. The primary focus of our work was to explore the binding of Asp₃, Asp and Asp₃ to calcite using a combined experimental and theoretical approach. The experiments clearly revealed that the additives exhibited an inhibitory effect on crystal growth, which increased asymptotically with the length of the peptide chain. The observation of a dead zone in the growth kinetics and the evaluation of the effectiveness factors confirmed a strong binding of the additives to the face active sites and the presence of a step-pinning mechanism of growth inhibition. This is supported by the quantitative analysis of the inhibition kinetics, which allows us to provide reliable estimates of the relatively large free energies of binding of the Asp, Asp; and Asp₃ to the growing crystal seed.

With the experimental data in hand, we were able to show that a basic force field approach results in significant over-binding of Asp to the flat calcite (104) surface. While this shortcoming was somewhat rectified by modifying certain inorganic-organic cross terms, the energetic effect of increasing the Asp content on the flat surface was completely opposite to that observed in experiment. Using a more elaborate surface model, however, resulted in a series of binding free energies with good qualitative and quantitative agreement with the experimental measurements. This result lends significant credibility to the underlying theoretical model and allows the investigation of binding properties with confidence.

Our results demonstrate, perhaps counterintuitively, that the most persistent binding feature of the Asp derivatives to calcite is the attachment of the positively charged ammonium group. This is frequently accompanied by simultaneous carboxylate attachment but always balanced by the solvation of one or more carboxylates in the aqueous phase. We presume that this balance is the primary factor responsible for the asymptotic growth of the binding energies as the number of Asp units is increased, a result also previously obtained in the context of inhibited growth (see Figure S12).

A probabilistic analysis shows that, for all three Asp derivatives, the additive prefers interactions with the edges and corners of the island than with the (104) surfaces in the center of the island or the bulk of the crystal. While many different structural motifs can be identified and classified, the overall indication is that the edges and corners are the preferred location for additive absorption. This, in turn, confirms that the growth inhibition mechanism relies primarily on the blocking of these sites, preventing further incorporation of dissolved ions and, hence, crystal growth.

ASSOCIATED CONTENT
Supporting Information
Sections S1-S11, Figures S1-S12, Tables S1-S6 (details on peptide synthesis, calcite seed preparation, growth kinetics, inhibition factor, simulation system, software, sampling protocols, force field, geometry of the binding modes and binding trends). The Supporting Information material is available free of charge on the ACS Publications website at http://pubs.acs.org.

AUTHOR INFORMATION
Corresponding Author
*E-mail: ana-suncana.smith@fau.de
*E-mail: Damir.Kralj@irb.hr
*E-mail: David.Smith@irb.hr

Author Contributions
§These authors contributed equally.

ACKNOWLEDGMENT
The Croatian Science Foundation is gratefully acknowledged for the financial support (project numbers: IP-11-2013–8238 (DS) and IP-11-2013-5055 (DK)). We also thank the Cluster of Excellence Engineering of Advanced Materials (EAM) and the Regionale Rechenzentrum Erlangen (RRZE) Friedrich–Alexander–Universität Erlangen–Nürnberg (FAU) for the computational resources. We thank Zlatko Brkljača (RBI) for assistance with construction of the calcite surfaces and Ivanka Jerić for helpful discussions in the early
stages of the project. We thank Radha D. Banhati for careful reading of the manuscript.

REFERENCES

1. Estroff, L. A. Introduction: Biominalarization. Chem. Rev. 2008, 108, 4329-4331.
2. DeOliveira, D. B.; Laursen, R. A. Control of Calcite Crystal Morphology by a Peptide Designed To Bind to a Specific Surface. J. Am. Chem. Soc. 1997, 119, 10627-10631.
3. Dickerson, M. B.; Sandhage, K. H.; Naik, R. R. Protein- and peptide-directed syntheses of inorganic materials. Chem. Rev. 2008, 108, 4935-4978.
4. Shen, J.-W.; Li, C.; van der Vegt, N. F. A.; Peter, C. Understanding the Control of Mineralization by Polyelectrolyte Additives: Simulation of Preferential Binding to Calcite Surfaces. J. Phys. Chem. C 2013, 117, 6904-6913.
5. Sand, K. K.; Pedersen, C. S.; Sjöberg, S.; Nielsen, J. W.; Makovicky, E.; Stipp, S. L. S. Biominalarization: Long-Term Effectiveness of Polyasaccharides on the Growth and Dissolution of Calcite. Cryst. Growth Des. 2014, 14, 5486-5494.
6. So, C. R.; Liu, J.; Fears, K. P.; Leary, D. H.; Golden, J. P.; Wahl, K. J. Self-Assembly of Protein Nanofibrils Orchestrates Calcite Step Movement through Selective Nonchiral Interactions. ACS Nano 2015, 9, 5782-5791.
7. Pai, R. K.; Pillai, S. Divalent cation-induced variations in polyelectrolyte conformation and controlling calcite morphologies: direct observation of the phase transition by atomic force microscopy. J. Am. Chem. Soc. 2005, 127, 13074-13078.
8. Addadi, L.; Weiner, S. Control and Design Principles in Biological Mineralization. Angew. Chem. Int. Ed. Engl. 1992, 31, 153-169.
9. Cusack, M.; Freer, A. Biominalarization: Elemental and Organic Influence in Carbonate Systems. Chem. Rev. 2008, 108, 4433-4454.
10. Espinosa, H. D.; Rim, J. E.; Barthelat, F.; Buehler, M. J. Merger of Minerals from Aqueous Solution: A New Model for the Calcite-Water Interface. J. Phys. Chem. C 2017, 121, 5533-5563.
11. Buljan Meić, I.; Kontrec, J.; Domazet Jurašin, D.; Njegić Džakula, B.; Štajner, L.; Lyons, D. M.; Dutour Sikirić, M.; Králj, D. Comparative Study of Calcium Carbonates and Calcium Phosphates Precipitation in Model Systems Mimicking the Inorganic Environment for Biominalarization. Cryst. Growth Des. 2017, 17, 1103-1117.
12. Montanari, G.; Lakshman, L. Z.; Tobler, D. J.; Dideriksen, K.; Dalby, K. N.; Bovet, N.; Stipp, S. L. S. Effect of Aspartic Acid and Glycine on Calcite Growth. Cryst. Growth Des. 2016, 16, 4813-4821.
13. Kubota, N.; Mullin, J. W. A kinetic model for crystal growth from aqueous solution in the presence of impurity. J. Cryst. Growth 1995, 152, 203-208.
14. Cabrera, N.; Vermilyea, D. A. The Growth of Crystal From Solution. Doremus, R. H.; Roberts, B. W.; Turnbull, D., Eds. Chapman & Hall: London, 1958, p. 393.
15. Chernov, A. A. The Spiral Growth of Crystals. Sov. Phys. Usp. 1961, 4, 116-148.
16. van Enkevort, W. J. P.; van den Berg, A. C. J. F. Impurity blocking of crystal growth: a Monte Carlo study. J. Cryst. Growth 1998, 183, 441-455.
17. Davis, K. J.; Dove, P. M.; De Yoreo, J. J. The Role of Mg2+ as an Impurity in Calcite Growth. Science 2000, 290, 1134-1137.
18. Reine, H. L.; Lin, T. W.; Kuo, S.; Kuo, T.-I.; Chao, T.-S.; Wang, Y.-H.; Su, C.-H. A Thermodynamically Consistent Force Fields for the Assembly of Inorganic, Organic, and Biological Nanostructures: The INTERFACE Force Field. Langmuir 2013, 29, 1754-1765.
19. Olsen, R.; Leirvik, K. N.; Kvanne, B.; Kuznetsova, T. Adsorption of Polyethylene Glycol on a Hydrated [10̅0̅4̅] Calcite Surface and Its Effect on Adsorbed Water. Langmuir 2015, 31, 8006-8017.
20. Raiteri, P.; Demichelis, R.; Gale, J. D.; Kellermeier, M.; Gebauer, D.; Quigley, D.; Wright, L. B.; Walsh, T. R. Exploring the influence of organic species on pre- and post-nucleation calcium carbonate. Faraday Discuss. 2012, 159, 61-85.
21. Ruiz-Agudo, E.; Di Tommaso, D.; Putnis, C. V.; de Leeuw, N. H.; Putnis, A. Interactions between Organophosphonate-Bearing Solutions and [10̅4̅] Calcite Surfaces: An Atomic Force Microscopy and First-Principles Molecular Dynamics Study. Cryst. Growth Des. 2010, 10, 3022-3035.
22. Sparks, D. J.; Romero-González, M. E.; El-Taboni, E.; Freeman, C. I.; Hall, S. A.; Kakanj, G.; Swanson, L.; Banwart, S. A.; Harding, J. H. Adsorption of poly acrylic acid onto the surface of calcite: an experimental and simulation study. Phys. Chem. Chem. Phys. 2015, 17, 23757-23763.
23. Harding, J. H.; Duffy, D. M. The challenge of biominalarizations to simulations. J. Mater. Chem. 2006, 16, 1105-1112.
24. Harding, J. H.; Duffy, D. M.; Sushko, M. L.; Rodger, P. M.; Quigley, D.; Elliot, J. A. Computational techniques at the organic-inorganic interface in biominalarization. Chem. Rev. 2008, 108, 4823-4854.
25. Demichelis, R.; Schuitemaker, A.; Garcia, N. A.; Koiari, K. B.; De La Pierre, M.; Raiteri, P.; Gale, J. D. Simulation of Crystallization of Biominerals. Annu. Rev. Mater. Res. 2018, 48, 327-352.
26. Schröder, K.-P.; Sauer, J.; Leslie, M.; Richard, C.; Catlow, A.; Thomas, J. M. Bridging hydrogel systems in zeolitic catalysts: a computer simulation of their structure, vibrational properties and acidity in protonated faujasites (H8Y zeolites). Chem. Phys. Lett. 1992, 188, 320-325.
27. Ponder, J. W.; Case, D. A. Force Fields for Protein Simulations. Adv. Protein Chem. 2003, 66, 27-85.
28. Freeman, C. L.; Harding, J. H.; Cooke, D. J.; Elliot, J. A.; Lardge, J. S.; Duffy, D. M. New forcefields for modeling biominalarization processes. J. Phys. Chem. 2007, 111, 11943-11951.
29. Pavese, A.; Catti, M.; Parker, S. C.; Wall, A. Modelling of the thermal dependence of structural and elastic properties of calcite, CaCO3. Phys. Chem. Miner. 1996, 23, 89-93.
30. Raiteri, P.; Gale, J. D.; Quigley, D.; Rodger, P. M. Derivation of an Accurate Force-Field for Simulating the Growth of Calcium Carbonate from Aqueous Solution: A New Model for the Calcite-Water Interface. J. Phys. Chem. C 2010, 114, 5997-6010.
31. Xiao, S.; Edwards, S. A.; Grätter, F. A New Transferable Forcefield for Simulating the Mechanics of CaCO3 Crystals. J. Phys. Chem. C 2011, 115, 20067-20075.
43. Wolthers, M.; Di Tommaso, D.; Du, Z.; de Leeuw, N. H. Calcite surface structure and reactivity: molecular dynamics simulations and macroscopic surface modelling of the calcite-water interface. Phys. Chem. Chem. Phys. 2012, 14, 15145-15157.

44. De La Pierre, M.; Raiteri, P.; Gale, J. D. Structure and Dynamics of Water at Step Edges on the Calcite \{10\bar{4}\} Surface. Cryst. Growth Des. 2016, 16, 5907-5914.

45. Nada, H. Difference in the Conformation and Dynamics of Aspartic Acid on the Flat Regions, Step Edges, and Kinks of a Calcite Surface: A Molecular Dynamics Study. J. Phys. Chem. C 2014, 118, 14335-14345.

46. Elhadj, S.; Salter, E. A.; Wierzbicki, A.; De Yoreo, J. J.; Han, N.; Dove, P. M. Peptide Controls on Calcite Mineralization: Polysapartate Chain Length Affects Growth Kinetics and Acts as a Stereocheminical Switch on Morphology. Cryst. Growth Des. 2005, 6, 197-201.

47. Ukrainczyk, M.; Kontrec, J.; Babić-Ivančić, V.; Brečević, L.; Kralj, D. Experimental design approach to calcium carbonate precipitation in a semicontinuous process. Powder Technol. 2007, 171, 192-199.

48. Brunauer, S.; Emmett, P. H.; Teller, E. Adsorption of Gases in Multimolecular Layers. J. Am. Chem. Soc. 1938, 60, 309-319.

49. Prunk, S.; Päll, S.; Schulz, R.; Larsson, P.; Bjelkmar, P.; Apostolov, R.; Shirts, M. R.; Smith, J. C.; Kasson, P. M.; van der Spoel, D.; Hess, B.; Lindahl, E. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics 2013, 29, 845-854.

50. Hub, J. S.; de Groot, B. L.; van der Spoel, D. g_wham—A Free Weighted Histogram Analysis Implementation Including Robust Error and Autocorrelation Estimates. J. Chem. Theory Comput. 2010, 6, 3713-3720.

51. Best, R. B.; Hummer, G. Optimized molecular dynamics force fields applied to the helix-coil transition of polypeptides. J. Phys. Chem. B 2009, 113, 9004-9015.

52. Lindorff-Larsen, K.; Piana, S.; Palmo, K.; Maragakis, P.; Klepeis, J. L.; Dror, R. O.; Shaw, D. E. Improved side-chain torsion potentials for the Amber ff99SB protein force field. Proteins 2010, 78, 1950-1958.

53. Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 1983, 79, 926-935.

TOC
On the Adsorption of Aspartate Derivatives to Calcite Surfaces in Aqueous Environment

Robert Stepić,†,‡ Lara Jurković,§ Ksenia Klementyeva,† Marko Ukraînczyk,)view Matija Gredičak,¶ David M. Smith,†,* Damir Kralj,§,* and Ana–Sunčana Smith†,‡,*

† Group for Computational Life Sciences, Division of Physical Chemistry, Ruđer Bošković Institute (RBI), Bijenička cesta 54, 10000 Zagreb, Croatia
‡ PULS Group, Institute for Theoretical Physics and Interdisciplinary Center for Nanostructured Films, FAU Erlangen–Nürnberg, Cauerstrasse 3, 91058 Erlangen, Germany
§ Laboratory for Precipitation Processes, Division of Material Chemistry, RBI, Bijenička cesta 54, 10000 Zagreb, Croatia
¶ Laboratory for Biomimetic Chemistry, Division of Organic Chemistry and Biochemistry, RBI, Bijenička cesta 54, 10000 Zagreb, Croatia

These authors contributed equally

Supporting Information
**S1. Peptide synthesis.** The (L-Asp)3 peptide was synthesized automatically from C- to N-terminal by the solid-phase Fmoc method on a commercially available Wang resin (Fluka, p-alkoxybenzyl alcohol resin, 200-400 mesh, 0.6-1.0 mmol/g resin) on a 0.1 mmol scale, by using the analytical grade L-Asp. The consecutive steps in the solid-phase peptide synthesis performed in each cycle were: (i) deprotection of Fmoc group by two treatments with 20 % piperidine in dimethylformamide (v/v); (ii) coupling by applying HBTU/HOBt/NMM activation and a three-fold excess of the appropriate Fmoc-amino acid for 1 h; (iii) removal of the peptides from the resin by treatment with a mixture of TFA–TIS–H2O in the ratio 9.5:0.25:0.25 (v/v) for 3 h. Successive deprotection and coupling steps were monitored by positive and negative Kaiser (ninhydrin) test, respectively. The peptide was obtained as a filtrate in trifluoroacetic acid (TFA) and precipitated with cold dry diisopropyl ether.

Analysis and purification of crude peptides was achieved by reversed-phase high-performance liquid chromatography (RP HPLC) performed on a Varian 940 LC system using a Eurospher 100 reversed-phase C-18 preparative (250 × 21.2 mm ID, 5 μm) (flow rate: 7.0 ml/min) or analytical (250 × 4.5 mm ID, 5 μm) (flow rate: 0.5 ml/min) column under isocratic conditions using 10 % MeOH in 0.1 % aqueous TFA (for solvent systems). UV detection was performed at 215 nm using a Varian 940 LC PDA dual array detector. The TFA ion present after preparative HPLC was removed using a SPE cartridge. The cartridge was first eluted with water and then with MeOH to recover peptide compounds. The eluent was evaporated and the residue dissolved in water and lyophilized. Peptides were at least 95 % pure as assessed by analytical RP HPLC. Molecular structure was confirmed by mass spectrometry and NMR spectroscopy. NMR spectra were recorded on a Bruker AV 600 spectrometer, operating at 150.91 MHz for 13C and 600.13 MHZ
for 1H nuclei. The spectra were measured in DMSO-d6 solutions at 25 °C. Chemical shifts in parts per million were referenced to TMS.

S2. Calcite Seed Crystals Preparation. Relatively large amount of structurally and morphologically well-defined rhombohedral calcite seed was prepared by previously described semi-continuous carbonation method in the Ca(OH)$_2$(s) – H$_2$O(l) – CO$_2$(g) system$^1$. Specifically, calcite seed has been prepared at constant concentration of dissolved calcium ions, $c_{\text{tot}} = 2.0$ mmol dm$^{-3}$, by controlling the addition of Ca(OH)$_2$ suspension ($c(\text{Ca(OH)}_2) = 100$ g dm$^{-3}$) into the thermostated bench-scale glass reactor, $V = 6.0$ dm$^3$. The mineralogical composition of the dried seed samples was analysed by FT-IR spectroscopy and by X-ray powder diffraction (Rigaku Ultima IV diffractometer, Cu Kα radiation, Ni filter, 5° Soller slits and proportional counter in Bragg- Brentano parafoocusing geometry). The morphology of the crystals was observed by scanning electron microscopy (SEM, JEOL GSM-7000F instrument). For the SEM observations, the dried samples were attached by carbon tape to an aluminium stub. The specific surface area was determined by the multiple BET method (Micromeritics, Gemini), using liquid nitrogen.

S3. Calcite crystal growth kinetics in the presence of additives. The calcite crystal growth kinetic experiments were performed in a thermostated double-walled glass vessel (400 cm$^3$). The metastable calcium carbonate solutions were prepared by mixing the equal volumes of CaCl$_2$ and NaHCO$_3$ solutions ($c = 5.0$ mmol dm$^{-3}$, pH$_i \approx 8.0$). The initial supersaturation of metastable solution was low, $S \approx 3.5$; $S = [(a(\text{Ca}^{2+}) a(\text{CO}_3^{2-}))/K_{\text{sp}}]^{1/2}$. An appropriate amount of the selected biomolecule (L-Asp, (L-Asp)$_2$ or (L-Asp)$_3$) was added into the bicarbonate solution before the mixing. The range of concentrations of biomolecules which caused the observable effects in the precipitation systems were initially determined and applied: the L-Asp varied in the range, $1.0$ mmol dm$^{-3} < c(\text{L-Asp}) < 25.0$ mmol dm$^{-3}$, the (L-Asp)$_2$ concentration was lower, $10.0$ µmol dm$^{-3}$
$c(L\text{-Asp})_2 < 250.0 \ \mu\text{mol dm}^{-3}$, as well as the concentration of $(L\text{-Asp})_3$, $0.5 \ \mu\text{mol dm}^{-3} < c(L\text{-Asp})_3 < 10.0 \ \mu\text{mol dm}^{-3}$.

The experiments were started by introducing 35.0 mg of previously prepared rhombohedral calcite seed crystals in the system ($A = 0.55 \ \text{m}^2 \ \text{dm}^{-3}$), immediately after mixing of the reactant solutions. The experiments were carried out at 25 °C and the systems were continuously stirred at a constant rate by means of a Teflon-coated magnetic stirring bar. The crystal growth process was followed by measuring the pH of the solution, using a combined glass-calomel electrode (Red Rod) and PHM 290, Radiometer (Figure S1). Samples of the suspension were periodically taken from the system in order to determine the total concentration of dissolved calcium by ion chromatography (ICS-1100, Dionex) and also to characterize the solid phase composition (ATR FT-IR, Tensor II, Bruker spectrophotometer). The chemicals used to prepare the reactant solutions, CaCl$_2$, NaHCO$_3$, NaOH, HCl, L -Asp and $(L\text{-Asp})_2$, were analytical grade and the deionized water was of high quality (conductivity < 0.055 µS cm$^{-1}$), while the $(L\text{-Asp})_3$ was synthesized by the protocol described previously.

Calculations of the solution composition (molar concentrations and activities of relevant ionic species), at any moment in the calcite crystal growth process, were based on continuous pH measurements and the known initial total concentrations of the reactants. The detailed calculation procedure, which takes into account the respective protolytic equilibria and equilibrium constants, as well as the charge and mass balance equations, is described previously.$^{2,3}$ The formation of respective ionic species of the used biomolecule has been considered. The crystal growth rate, $R$, was calculated by numerical differentiation of the total dissolved calcium concentration, $Ca_{\text{tot}}$, as a function of time $t$, and normalized with respect to the surface area of the precipitate, $A$, at a particular moment: $R = \frac{-dCa_{\text{tot}}}{dt \cdot A}$. During the growth of calcite crystals, the total surface area

\[S5\]
increases, which was also considered in calculations and was calculated by using the data on the concentration of precipitated total calcium carbonate.

**Figure S1.** Variation of pH with different concentrations of Asp (top) and Asp₂ (bottom).

Figure S2 shows the growth rates obtained for model systems and for the systems containing different additives, plotted as a function of relative supersaturation. It is evident from the values of critical supersaturation, S*, obtained for comparable additive concentrations, that the inhibition
efficiency is significantly different: (Asp)$_3$ $\gg$ (Asp)$_2$ $\gg$ Asp. The controlling growth mechanism of calcite in the model system, but also in the system containing additive, have been determined by testing the appropriate crystal growth models. As could be expected for the low-supersaturated systems, the growth on the spiral dislocation, described by the rate law, $R = k_s(S - 1)\cdot \ln S$, has been

**Figure S2.** Crystal growth rate of calcite as a function of relative supersaturation in the systems containing different concentrations of additives. The lines are consistent with a rate law of the form: $R = k_s(S - 1)\cdot \ln S$. The curves corresponding to the additive-free systems are shown as solid bold lines.
observed and the appropriate value of the rate constant, \( k_s = 2.0 \pm 0.02 \text{ µmol dm}^{-3} \text{ m}^{-2} \text{ s}^{-1} \), was found. The results are consistent with values obtained for calcite growth at similar experimental conditions.\(^1\)\(^2\)

**S4. Growth inhibition factor.** The decreased of the growth inhibition factor with increasing supersaturation (Figure S3) is consistent with the model which correlates the size of the critical surface nucleus and the separation of the active sites at the crystal surfaces available for impurity adsorption.

![Figure S3](image)

**Figure S3.** Growth inhibition factor, \( \alpha \), as a function of supersaturation for different biomolecular additives.

**S5. Simulation system.** The calcite slab employed is approximately 2.2 nm thick. The corresponding surface area is 4.7 x 4.7 nm\(^2\) and the slab contains 960 calcium carbonate units. The island is constructed following the procedure already reported in previous work,\(^4\) where 16 calcium carbonate pairs were cut from the bottom layer and put on the topmost layer forming a rhombus shaped island with 5 distinct features. The features of the island are a calcium corner, acute carbonate corner, acute carbonate edge, obtuse carbonate corner, and obtuse carbonate edge. Above the calcite surface we have placed a 7.7 nm layer containing 6000 water molecules, which
reproduces the ambient water density in bulk. On top of the water, we have included an 11 nm thick vacuum, to prevent strong ordering of water in the upper layer induced by the bottom surface of the calcite slab. For balanced results, it is essential to construct a water layer thick enough that at some point it reproduces bulk properties, non-perturbed by the presence of the two enclosing interfaces. Lastly, the (poly) amino acids in our study are immersed into the water completing the spatial description. In all cases, the side-chain carboxylate groups were used in the ionized (CO$_2^-$) forms, as were the N- and C-terminal ends (H$_3$N$^+$- and -CO$_2^-$) of each Asp-derivative, in accordance to expectations at neutral pH (Scheme 1 of the manuscript). An illustration of the system described above is shown in Figure S4.
S6. Software. All the molecular dynamics simulations were performed using GROMACS 4.5.5. Quantum mechanical optimizations were done in Gaussian09. RESP fitting of charges and Amber parameter extraction was carried out with AmberTools 2016. Optimization of the crystal structures and parameter fitting was done using the General Utility Lattice Program (GULP). For visualization and analysis of produce trajectories we used Visual Molecular Dynamics (VMD).

S7. Simulation details. Fully atomistic molecular dynamics simulations were performed. We work in the canonical ensemble, keeping the number of particles, volume and the temperature (300 K) constant. Temperature control was exerted through stochastic dynamics with the coupling time-constant of 0.1 ps. Long-range interactions were resolved using the particle mesh Ewald approach where the cutoff of 1.2 nm was used in the direct space sum. Short range non-bonding interactions were calculated using a cutoff of 1.2 nm. In the simulations, non-interacting layers at the bottom of the calcite slab were kept frozen to reproduce the bulk properties of the crystal better. Bonds with hydrogen were constrained using the LINCS algorithm. Prepared systems were minimized using the steepest descent method and then equilibrated for 200 ps.
The relevant biomolecule was pulled to the surface, equilibrated there for 20 ps, and then pulled away from the surface along the z-direction, perpendicular to the surface. During the pulling, the COM of the amino acid was attached to a moving virtual particle. The spring constant of the attachment was 1000 kJ mol$^{-1}$ nm$^{-2}$ and the rate of movement of the virtual particle was 0.01 nm/ns. From the 400 ps long pull trajectory, 21 system configurations were extracted. Each of those configurations had a different distance between the COM of the amino acid and the COM of the frozen part of the calcite slab. There were 16 equidistant configurations for the biomolecule residing in the vicinity of the surface characterized by increasing the separation by 0.04 nm between COMs for each successive configuration, and 5 configurations where the biomolecule was further away from the surface for which the separation between COMs increased by 0.1 nm. Those configurations were starting points for umbrella sampling simulations using harmonic bias potentials. The overall protocol thus ensures sufficient sampling of a geometric region extending up to 1.2 nm from the surface. This allowed for construction of free energy profiles with a well-defined water bulk in which the organic molecule no longer interacts with the inorganic surface.

**Figure S5.** A set of histograms from a single umbrella sampling simulation. Closer to the surface higher restraint was applied to the center of mass of biomolecule (red), while for biomolecule further away from the surface lower restraint was used (blue).
In the biased sampling simulations, the force constant was set to 500 kJ mol$^{-1}$ nm$^{-2}$ for all configurations where the biomolecule is further away from the surface and a value of 7000 kJ mol$^{-1}$ nm$^{-2}$ was used close to the surface. All the sampling configurations were propagated for 8 ns. For analysis purposes, the data from the first ns was omitted. This resulted in the distributions of the positions of COM of the biomolecule that were collected into separate histograms for each window. An example of such a collection of histograms is shown in Figure S5. If we add up the simulation time over 21 windows, we obtain a total of 147 ns of sampling time for each umbrella simulation. To obtain unbiased distribution and potentials of mean force (PMF) from the histograms, we used the weighted histogram analysis method (WHAM). Convergence for all obtained distributions was checked by calculating PMFs for the first 5, 6 and 7 ns. Island sampling required significantly larger amount of data, as each feature needs to be sampled separately. To improve statistics, we added an additional run which starts in the middle of the island. Each individual run produced 147 ns of data, which amounted to 882 ns of data per biomolecule.

**S8. Force Field.** Calcite charges and non-bonding parameters for the potential of the Buckingham type, as well as the bonding parameters, were taken from work by Raiteri. Raiteri et al. calcite force field was constructed with a focus on reproducing the free energies of solvation of calcium and carbonate ions, and consequently much more attention was devoted to having a proper description of the interface with water. Since our goal is to provide calculations of thermodynamic potentials, such as the Helmholtz free energies of binding, it is crucial to have a consistent description of the crystal-water interface to account for the entropic contribution of displacement of water to the free energies as precisely as possible. The water model used is TIP3P. Biomolecular parameters for intramolecular interactions and interactions with water were taken
from Amber ff99sb-ILDN\textsuperscript{16} force field (Lennard-Jones type). The expression that we utilized for this interaction between atoms $i$ and $j$ is given by the following equation:

$$E_{ij} = \frac{A_{ij}}{r^{12}} + \frac{B_{ij}}{r^{6}}$$

Parameters for interactions between the biomolecule and the calcium were obtained using an approach originally developed and used for zeolites by Schroeder et al. \textsuperscript{17}, while the interactions with carbonates were taken from Amber ff99sb-ILDN following a slightly modified approach proposed by Freeman et al. \textsuperscript{18}. The difference from methodology proposed by Freeman is that also crystal oxygen-biomolecule interaction is extracted from Amber, rather than employing combination rules. The parameters obtained in this way for Asp, Asp\textsubscript{2} and Asp\textsubscript{3} are shown in Tables S1-S3. The effect of using this kind of cross interaction instead of the standard Freeman methodology is showcased on the case of aspartate in Figure S6. We were using potentials of Buckingham and Lennard-Jones type simultaneously, avoiding the need to do additional fitting. Parameters for the interaction of water with the surface were taken from the article introducing the inorganic force field.\textsuperscript{14} Point charges for zwitterionic aspartate derivatives, which are not included in the used Amber variant, were obtained using the RESP fitting procedure after the optimization on MP2/6-31G(d)\textsuperscript{19} level and electrostatic potential calculation on HF/6-31G(d) level. The optimization was done in polarizable continuum ($\varepsilon = 80$) in order to screen strong Coulomb interactions and prevent the proton transfer that would otherwise occur in the zwitterion.

\textbf{Table S1.} Interaction parameters of oxygen from the crystal with every atom of Asp
Table S2. Interaction parameters of oxygen from the crystal with every atom of Asp$_2$

| Crystal | Biomolecule | B / kJ mol$^{-1}$ nm$^6$ | A / kJ mol$^{-1}$ nm$^{12}$ |
|---------|-------------|-------------------------|---------------------------|
| Oc      | N3          | 2.83349E-03             | 2.53897E-06               |
| Oc      | H           | 6.42266E-05             | 4.29258E-09               |
| Oc      | CT          | 2.62220E-03             | 2.71057E-06               |
| Oc      | HP          | 2.12946E-04             | 4.71874E-08               |
| Oc      | HC          | 4.67792E-04             | 2.27716E-07               |
| Oc      | C           | 2.32491E-03             | 2.40326E-06               |
| Oc      | O2          | 2.36347E-03             | 1.58939E-06               |
| Oc      | OP          | 2.36347E-03             | 1.58939E-06               |

Table S3. Interaction parameters of oxygen from the crystal with every atom of Asp$_3$

| Crystal | Biomolecule | B / kJ mol$^{-1}$ nm$^6$ | A / kJ mol$^{-1}$ nm$^{12}$ |
|---------|-------------|-------------------------|---------------------------|
| Oc      | N3          | 2.83349E-03             | 2.53897E-06               |
| Oc      | H           | 6.42266E-05             | 4.29258E-09               |
| Oc | CT   | E0   | E6   |
|----|------|------|------|
| Oc | HP   | 2.12946E-04 | 4.71874E-08 |
| Oc | HC   | 4.67792E-04 | 2.27716E-07 |
| Oc | C6   | 2.32491E-03 | 2.40326E-06 |
| Oc | O    | 2.36347E-03 | 1.58939E-06 |
| Oc | N    | 2.83349E-03 | 2.53897E-06 |
| Oc | H1   | 3.85425E-04 | 1.54585E-07 |
| Oc | C    | 2.32491E-03 | 2.40326E-06 |
| Oc | O2   | 2.36347E-03 | 1.58939E-06 |
| Oc | ON   | 2.36347E-03 | 1.58939E-06 |
| Oc | OK   | 2.36347E-03 | 1.58939E-06 |
| Oc | OS   | 2.36347E-03 | 1.58939E-06 |

**S9. Flat Surface Binding.** The obtained free energy profile (Figure S7) can be dissected to extract the atomistic details from the observed minima. Three different bound states are found for Asp, Asp2 and Asp3 representing the global minima on the respective potentials of mean force on the flat (104) surface. We observe that the dominant mode of binding of aspartate to the flat surface is achieved through the zwitterionic pair, while the remaining carboxylate group stays solvated (Figure S8a). Same pattern can be observed for Asp2 and Asp3 as well (Figure S8b and Figure S8c), where the amino group is interacting with the carbonate in the crystal and there is an additional, albeit weaker, interaction through one of the carboxylate groups of the peptide.
Figure S6. Potential of mean force for the process of binding of aspartate to the flat (104) surface of calcite. Effect of the proposed modification of the Freeman method on the PMF with Raiteri calcite force field.

Figure S7. Potentials of mean force for Asp, Asp$_2$ and Asp$_3$ on the (104) surface of calcite showcasing a decreasing trend in binding energy with an increase in peptide chain length.

Figure S8. Dominant modes of binding of Asp, Asp$_2$, and Asp$_3$ to the (104) surface of calcite corresponding to the global minima on the PMFs shown in Figure S7.
**S10. Island Binding.** For the analysis we used the eight lowest lying umbrella windows for each feature, effectively covering 3.2 Å along the reaction coordinate. In those windows we calculated the distance of the COM of Asp, Asp$_2$ and Asp$_3$ from each individual feature. The collective data for all the features is then used to determine where the biomolecule spends the most time, and in the process the island feature(s) which contribute to the binding the most are identified. We used empirical binding cutoff values of 7 Å, 8.5 Å and 10 Å for Asp, Asp$_2$ and Asp$_3$, respectively. Those values were used because they are significantly larger than half of the distance from the N-terminal to C-terminal ends for individual biomolecules (~2.5 Å for Asp, ~4 Å for Asp$_2$, ~5.5 Å for Asp$_3$). If the distance to all of the features exceeded this value, it is reasonable to consider the biomolecule not bound to the island. Otherwise, we said that the molecule is interacting with the closest island feature. Following this approach, we were able to classify the biomolecule in each frame to one of the seven classes: bound to acute carbonate, bound to obtuse carbonate, bound to acute edge, bound to obtuse edge, bound to calcium, bound to the middle of the island or unbound.
Figure S9. Most likely bound geometries of Asp to the specific features of the island, based on statistical analysis (Table S4).

Figure S10. Most likely bound geometries of Asp$_2$ to the specific features of the island, based on statistical analysis (Table S5).
Figure S11. Most likely bound geometries of Asp$_3$ to the specific features of the island, based on statistical analysis (Table S6).

Table S4. Average distance (nm) of Asp groups (nitrogen in the N-terminal amino group (ZN), carbon in the C-terminal carboxylic group (ZC), carbon in the carboxylate side chain (SC)) from the surface. Umbrella windows corresponding to the global minimum in the individual feature PMFs (not shown here) were used in the analysis, and the abbreviations used correspond to those found in Figure 4 in the main text.

| FEATURES | ZN  | ZC  | SC  |
|----------|-----|-----|-----|
| AC       | 0.61| 0.78| 0.87|
| AE       | 0.53| 0.53| 0.70|
| OC       | 0.34| 0.33| 0.56|
| OE       | 0.35| 0.48| 0.71|
| CA       | 0.33| 0.51| 0.58|
| MI       | 0.61| 0.76| 0.80|
Table S5. Average distance (nm) of Asp₂ groups (nitrogen in the N-terminal amino group (ZN), carbon in the C-terminal carboxylic group (ZC), nitrogen forming the peptide bond (PN), carbon forming the peptide bond (PC), carbons in the carboxylate side chain (SC1 and SC2)) from the surface. Umbrella windows corresponding to the global minimum in the individual feature PMFs (not shown here) were used in the analysis, and the abbreviations used correspond to those found in Figure 4 in the main text.

| FEATURE | ZN  | ZC  | PN  | PC  | SC1 | SC2 |
|---------|-----|-----|-----|-----|-----|-----|
| AC      | 0.60| 1.04| 0.84| 0.79| 0.81| 0.97|
| AE      | 0.54| 0.89| 0.76| 0.71| 0.63| 0.88|
| OC      | 0.62| 0.94| 0.79| 0.76| 0.79| 0.70|
| OE      | 0.64| 1.05| 0.87| 0.83| 0.79| 0.84|
| CA      | 0.62| 0.74| 0.72| 0.77| 0.77| 0.87|
| MI      | 0.60| 1.03| 0.82| 0.77| 0.66| 0.78|

Table S6. Average distance (nm) of Asp₃ groups (nitrogen in the N-terminal amino group (ZN), carbon in the C-terminal carboxylic group, nitrogen forming the peptide bond (PN), carbon forming the peptide bond (PC), carbons in the carboxylate side chain (SC1 and SC2)) from the surface. Umbrella windows corresponding to the global minimum in the individual feature PMFs (not shown here) were used in the analysis, and the abbreviations used correspond to those found in Figure 4 in the main text.

| FEATURE | ZN  | ZC  | PN1 | PC1 | PN2 | PC2 | SC1 | SC2 | SC3 |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| AC      | 0.32| 0.83| 0.48| 0.48| 0.71| 0.63| 0.54| 0.57| 0.91|
| AE      | 0.63| 0.48| 0.53| 0.65| 0.53| 0.50| 0.85| 0.33| 0.77|
| OC      | 0.33| 0.95| 0.62| 0.54| 0.82| 0.76| 0.50| 0.95| 0.78|
| OE      | 0.82| 0.80| 0.80| 0.83| 0.83| 0.80| 0.64| 0.91| 0.83|
| CA      | 0.32| 0.51| 0.42| 0.35| 0.39| 0.46| 0.61| 0.54| 0.32|
| MI      | 0.60| 0.86| 0.84| 0.79| 0.90| 0.85| 0.85| 1.13| 0.85|
S11. Binding Trends.

![Absolute Binding Energy vs N(Asp)](image)

**Figure S12.** Comparison of the absolute binding energies from experimental measurements (Exp) and calculated values (Calc), in this work, with the previous measurements of Montanari et al.\(^{20}\) and the semi-empirical calculations of Elhadj et al.\(^{21}\)

S12. References

1. Ukrainczyk, M.; Kontrec, J.; Babić-Ivančić, V.; Brečević, L.; Kralj, D. Experimental design approach to calcium carbonate precipitation in a semicontinuous process. *Powder Technol.* 2007, *171*, 192-199.
2. Ukrainczyk, M.; Gredičak, M.; Jerić, I.; Kralj, D. Interactions of salicylic acid derivatives with calcite crystals. *J. Colloid Interface Sci.* 2012, *365*, 296-307.
3. Ukrainczyk, M.; Gredičak, M.; Jerić, I.; Kralj, D. Interactions of Scalenohedral Calcite Crystals with Acidic Amino Acid Derivatives of Salicylic Acid. *Cryst. Growth Des.* 2014, *14*, 4335-4346.
4. Wolthers, M.; Di Tommaso, D.; Du, Z.; de Leeuw, N. H. Calcite surface structure and reactivity: molecular dynamics simulations and macroscopic surface modelling of the calcite-water interface. *Phys. Chem. Chem. Phys.* 2012, *14*, 15145-15157.
5. Pronk, S.; Páll, S.; Schulz, R.; Larsson, P.; Bjelkmar, P.; Apostolov, R.; Shirts, M. R.; Smith, J. C.; Kasson, P. M.; van der Spoel, D.; Hess, B.; Lindahl, E. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics* 2013, *29*, 845-854.
6. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Li, P.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian09, Revision A02, Gaussian, Inc.: Wallingford CT, 2016.

7. Case, D. A.; Betz, R. M.; Cerutti, D. S.; Cheatham, T. E.; Darden, T. A.; Duke, R. E.; Giese, T. J.; Gohlke, H.; Goetz, A. W.; Homeyer, N.; Izadi, S.; Janowski, P.; Kaus, J.; Kovalenko, A.; Lee, T. S.; LeGrand, S.; Li, P.; Lin, C.; Luchko, T.; Luo, R.; Madej, B.; Mermelstein, D.; Merz, K. M.; Monard, G.; Nguyen, H. N.; Nguyen, H. T.; Omelyan, I.; Onufriev, A.; Roe, D. R.; Roitberg, A.; Sagui, C.; Simmerling, C. L.; Botello-Smith, W. M.; Swails, J.; Walker, R. C.; Wang, J.; Wolf, R. M.; Wu, X.; Xiao, L.; Kollman, P. A. AMBER 2016, University of California: San Francisco, 2016.

8. Gale, J. D.; Rohl, A. L. The General Utility Lattice Program (GULP). Mol. Simul. 2003, 29, 291-341.

9. Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual molecular dynamics. J. Mol. Graph. 1996, 14, 33-38.

10. Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An N-log(N) method for Ewald sums in large systems. J. Chem. Phys. 1993, 98, 10089.

11. Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. A smooth particle mesh Ewald method. J. Chem. Phys. 1995, 103, 8577.

12. Hub, J. S.; de Groot, B. L.; van der Spoel, D. g_wham—A Free Weighted Histogram Analysis Implementation Including Robust Error and Autocorrelation Estimates. J. Chem. Theory Comput. 2010, 6, 3713-3720.

13. Raiteri, P.; Demichelis, R.; Gale, J. D.; Kellermeier, M.; Gebauer, D.; Quigley, D.; Wright, L. B.; Walsh, T. R. Exploring the influence of organic species on pre- and post-nucleation calcium carbonate. Faraday Discuss. 2012, 159, 61-85.

14. Raiteri, P.; Gale, J. D.; Quigley, D.; Rodger, P. M. Derivation of an Accurate Force-Field for Simulating the Growth of Calcium Carbonate from Aquous Solution: A New Model for the Calcite–Water Interface. J. Phys. Chem. C 2010, 114, 5997-6010.

15. Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 1983, 79, 926-935.

16. Best, R. B.; Hummer, G. Optimized molecular dynamics force fields applied to the helix-coil transition of polypeptides. J. Phys. Chem. B 2009, 113, 9004-9015.

17. Schröder, K.-P.; Sauer, J.; Leslie, M.; Richard, C.; Catlow, A.; Thomas, J. M. Bridging hydrolyl groups in zeolitic catalysts: a computer simulation of their structure, vibrational properties and acidity in protonated faujasites (HxY zeolites). Chem. Phys. Lett. 1992, 188, 320-325.
18. Freeman, C. L.; Harding, J. H.; Cooke, D. J.; Elliott, J. A.; Lardge, J. S.; Duffy, D. M. New forcefields for modeling biomineralization processes. *J. Phys. Chem. C* 2007, 111, 11943-11951.
19. Møller, C.; Plesset, M. S. Note on an Approximation Treatment for Many-Electron Systems. *Phys. Rev.* 1934, 46, 618-622.
20. Montanari, G.; Lakshtanov, L. Z.; Tobler, D. J.; Dideriksen, K.; Dalby, K. N.; Bovet, N.; Stipp, S. L. S. Effect of Aspartic Acid and Glycine on Calcite Growth. *Cryst. Growth Des.* 2016, 16, 4813-4821.
21. Elhadj, S.; Salter, E. A.; Wierzbicki, A.; De Yoreo, J. J.; Han, N.; Dove, P. M. Peptide Controls on Calcite Mineralization: Polyaspartate Chain Length Affects Growth Kinetics and Acts as a Stereochemical Switch on Morphology. *Cryst. Growth Des.* 2005, 6, 197-201.
