Phage Display Screening as a Rational Approach to Design Additives for Selective Crystallization Control in Construction Systems

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The design of additives showing strong and selective interactions with certain target surfaces is key to crystallization control in applied reactive multicomponent systems. While suitable chemical motifs can be found through semi-empirical trial-and-error procedures, bioinspired selection techniques offer a more rationally driven approach and explore a much larger space of possible combinations in a single assay. Here, phage display screening is used to characterize the surfaces of crystalline gypsum, a mineral of broad relevance for construction applications. Based on next-generation sequencing of phages enriched during the screening process, a triplet of amino acids, DYH, is identified as the main driver for adsorption on the mineral substrate. Furthermore, oligopeptides containing this motif prove to exert their influence in a strictly selective manner during the hydration of cement, where the sulfate reaction (initial setting) is strongly retarded while the silicate reaction (final hardening) remains unaffected. In the final step, these desired additive characteristics are successfully translated from the level of peptides to that of scalable synthetic copolymers. The approach described in this work demonstrates how modern biotechnological methods can be leveraged for the systematic development of efficient crystallization additives for materials science.

1. Introduction

Crystallization is an elementary phenomenon with broad relevance across a wide spectrum of scientific disciplines, ranging all the way from natural biomineralization[1] to industrial applications.[2] Steering the course and outcome of a crystallization process is key to the design and production of materials with specific desired properties.[3] A common concept to influence a crystallizing system is the use of soluble (macro)molecules as additives, which interact with the forming solid phase during its nucleation, growth and further ripening.[4] Obviously, any such effects require the additive to exhibit a reasonably strong affinity to bind to or adsorb on the surface of relevant species such as nucleated nanoparticles or growing crystal facets. This is all the more true for crystallization in multicomponent systems, where the ability to address certain surfaces/phases in a more or less selective manner represents both a major challenge and a long-standing goal. In this context, one prominent case is ordinary Portland cement, which comprises a number of different inorganic phases that simultaneously react with water to yield various hydration products providing the resulting concrete with the desired strength and durability.[3,5] The need for selective crystallization control can readily be illustrated in such systems by a simple example. In
order to extend the time window during which cement paste retains its workability, the hydration of calcium sulfate components (responsible for early setting) must be retarded, whereas the later reaction of siliceous components to calcium silicate hydrate (C-S-H) should remain unaffected so that final hardening does not take too long. Hence, crystallization additives are required that interact strongly with CaSO₄ phases, but at the same time do not show significant affinity to C-S-H species.

In the past, the search for additives with selective effects in cementitious and other applied systems has been guided largely by chemical intuition and/or empirical screening. Although such approaches can lead to success, the sheer infinite number of possible structural motifs and combinations thereof call for rational concepts to identify optimized additive chemistries for the given target phase or surface. This task, as many others, has been mastered by Nature through an evolutionary selection of complex proteins, which for instance enable precise control over polymorphism in mineralized textures of living organisms such as mollusk shells. With the advent of modern techniques to emulate natural selection in the laboratory, most notably directed evolution and phage display (PD) screening, it became possible to pick ideal (peptide-based) structures out of libraries of millions or billions of variants for a given purpose. While these techniques were originally developed to engineer enzymes for biocatalysis or tune biomolecular interactions in life science applications, their potential has also been recognized and exploited in the field of materials research. Among many other examples, directed evolution was successfully used to design protein domains with strong and selective affinity for different plastics, while phage display was applied to derive peptide-based binding motifs for prominent minerals like calcium carbonate or calcium phosphate, as well as various other materials such as polymers, semiconductors or metals. In the context of cementitious systems, a PD screening approach was employed to derive interaction patterns on C-S-H surfaces, which were later exploited to prepare mesocrystalline elastic concrete.

In the present work, we have performed phage display screening to characterize the surfaces of gypsum (calcium sulfate dihydrate), an important inorganic mineral of high abundance in natural environments and broad industrial use as a binder in mortars, plasters, and cements. Based on the observed selection patterns, a peptide binding motif is identified which not only shows strong adsorption on gypsum surfaces but also provides selective control over the crystallization of the mineral under reactive conditions in complex systems. Finally, the binding motif has been translated to the level of synthetic copolymers, in order to leverage the gained knowledge for the development of new and scalable additive technologies.

2. Results and Discussion

Interaction patterns across the interfaces of crystalline gypsum particles and water were explored using conventional combinatorial libraries of M13 bacteriophages, which contain ca. 10⁹ variants of randomized dodecapeptides fused to the phage surface, in overall three selection cycles (so-called panning rounds, see Figure S1, Supporting Information for more details). After each panning round, the phages enriched in the selected library by preferred binding to the gypsum surfaces were investigated with respect to their terminal oligopeptide chemistry by next-generation sequencing (NGS), which delivered more than a million sequences that were reviewed and analyzed statistically. The results of these studies are summarized in Figure 1 and Table 1. Surprisingly, a distinct enrichment of one single peptide sequence (DYHDPSLPTLRK, see Figure S2, Supporting Information) occurred already after the first selection.

![Figure 1. Phage display screening on gypsum. a) AFM height image showing filamentous M13 bacteriophages adsorbed on a crystalline gypsum surface. b) Schematic drawing of a bacteriophage, with the fused terminal dodecapeptides highlighted in red (reproduced with permission). c) Diagram showing the fraction of the eight most frequent dodecapeptides found after each panning round.](image-url)

![Table 1. Detailed results of phage display screening on gypsum, with the eight most frequently found peptide sequences (in single-letter amino acid code) after each panning round and the total number of sequences analyzed by NGS.](table-url)
cycle, which indicates effective adsorption on the gypsum surfaces with a strong preference for this motif. As expected, the identified lead sequence becomes more dominant in the following two panning rounds and accounts for more than 80% of the phage library after the third selection cycle. The affinity of the enriched family of phages to the surface of crystalline gypsum is directly evident in height images acquired by atomic force microscopy from the [120] face of a macroscopic gypsum crystal (Figure 1a), which was incubated with the library after the third panning round and shows numerous adhering filamentous phages (estimated coverage: ca. 10¹⁰ per cm²) despite extensive rinsing.

To further verify the results of our PD screenings, different dodecapeptides were synthesized (marking the transition from a biological system to one where only the chemical and physical interactions of selected oligomeric peptides remain) and investigated with respect their affinity to adsorb on conventional gypsum powder using aqueous dispersions at neutral pH (i.e., conditions close to those experienced by the phages in the actual screenings). The results of these measurements are compared in Figure 2. Indeed, the peptide with the lead sequence from the PD screening (DYHDPSLPTLRK, molecular weight (MW) = 1442 Da) showed significantly stronger adsorption on gypsum than, for example, peptide binders identified in previous studies for other calcium-bearing minerals like apatite (SVSVGMKPSPRP, MW = 1241 Da) or C-S-H (LPNHLAGILRAD, MW = 1290 Da). To exclude the possibility of simple overexpression of the lead motif in the panning cycles, dodecapeptides with sequences TAKYLMRPGPPL and QVNGLgersQQM were also synthesized and tested for CaSO₄ affinity, which proved to be significantly lower (data not shown) and thus confirmed the high preference for DYHDPSLPTLRK. However, the absolute adsorbed amounts (q_ads) for the latter dodecapeptide were still rather low (0.03 mg g⁻¹) as compared to known organic molecules with high (but presumably unspecified) affinity to gypsum such as poly(acrylic acid) (q_ads = 0.13 mg g⁻¹ for PAA with MW = 2500 Da under the given conditions). Therefore, different segments of the lead peptide sequence (e.g., amino acid triplets) were evaluated separately with respect to their propensity to bind to gypsum surfaces. These analyses revealed that the terminal triplet of the lead 12-mer sequence, that is, DYH or Asp-Tyr-His, governs the interaction of the enriched phages with the inorganic substrate. While a single DYH trimer adsorbs only weakly on gypsum particles, the affinity increases steeply for oligomers with multiple repetitive DYH units and reaches a maximum for the corresponding dodecamer (i.e., (DYH)₁ ≪ (DYH)₂ ≪ (DYH)₄ > (DYH)₈), probably due to unfavorable folding processes within the peptide structure at higher molecular weights (as supported by circular dichroism (CD) spectroscopy, see Figure S3, Supporting Information). Most notably, adsorption of the (DYH)₈ dodecamer (MW = 1680 Da) on gypsum is significantly stronger (q_ads = 0.23 mg g⁻¹) than for a poly(acrylic acid) with similar molecular weight (q_ads = 0.13 mg g⁻¹ for MW = 2500 Da). Moreover, any permutations of the identified acid triplet at a fixed length of 12 amino acids decreased the adsorbed amounts to a greater or lesser extent (i.e., (DYH)₁ > (DDYYHH)₁ > D₁Y₁H₈), indicating that local amino acid sequence modulates the binding mechanism (although the sheer presence of D, H, and Y appears to be the main criterion for strong adsorption).

These observations, as well as analogous adsorption experiments using different parts of the full-length sequence (data not shown), suggest that the bacteriophages carrying the lead peptide dodecamer bind “heads-on” to the gypsum surface(s) via their terminal DYH triplet. Although the influence of the other 8 amino acids in the strongly enriched lead sequence remains uncertain, it seems reasonable to assume that further subtle, but specific effects – caused by the peptide and/or the mineral surface – are at play to account for the selection of this triplet of amino acids.

To shed further light on the binding mechanism of the DYH motif on gypsum/water interfaces, we have performed molecular dynamics (MD) simulations (due to the lack of experimental techniques allowing peptide conformation at these interfaces to be studied on a molecular level). Among the different faces of crystalline gypsum, significant adsorption was only observed on {120} (ΔG_ads = -2.5 kcal mol⁻¹), which constitutes a dominant part of the habit of typical (equilibrium) morphologies and is relatively hydrophobic. On the other main face, {010}, as well as on the usually fast-growing {−111} face, no distinct interaction was found while the second “reactive” face, {011}, even showed slight repulsion against the model peptide structure (see Table S1, Supporting Information). These results can be understood structurally by analyzing the molecular adsorption behavior observed during the simulation, as illustrated by selected MD snapshots from the trajectory in Figure 3. Mostly, different bidentate binding occurred, involving either one oxygen of the carboxylate moiety of aspartic acid to calcium and the hydroxyl group of tyrosine to sulfate, or both oxygens of Asp to two different calcium ions on the mineral substrate. In addition, the OH group of Tyr was found to bind to water present on the surface, enabling multidentate binding.
patterns as shown in the lower snapshot of Figure 3. Histidine generally plays a tangential role in the observed interactions. It should be noted, however, that the His moieties were chosen to be neutral in the simulations, whereas any residual (local) protonation could enhance interactions with the negatively charged sulfate groups. In this case, pairs of mutually matching partners could exist at the (120) surface (i.e., a binding "triad" of Asp with Ca^{2+}, His with SO_4^{2-}, and Tyr with H_2O) and explain the experimentally observed high affinity of the peptide motif to these faces via crystal lattice recognition. By contrast, adsorption of the peptide motif DYH on the (120) face of crystalline gypsum, as derived from MD simulations in stick-figure representation. The affinity of the triplet to adsorb on the surface was enhanced by the addition of two (inert) methylacrylate units on both ends of the peptide. Water molecules within a radius of 3 Å around the peptide and the mineral surface are shown to indicate the local hydrogen bonding network. Color code: nitrogen (blue), hydrogen (white), oxygen (red), carbon (green), calcium (green), sulfate (brown-yellow), and crystal water (white-pale red). Note the multidentate binding mode in the lower image, which involves both oxygen atoms of the carboxylate moiety, another amide oxygen as well as the phenolic hydroxy group through a water-mediated type of interaction.

Having confirmed and characterized the preferential binding of the PD-derived peptide motif on gypsum surfaces in model systems, we now turn to consider the potential of the lead sequences to control crystallization selectively under reactive and application-relevant conditions. To this end, the hydration of cement with different clinker compositions was monitored in situ using heat-flow calorimetry, in the presence and absence of the full-length peptide DYHDPSLPTLRK and the replicated local motif (DYH)_4. For comparison, neat poly(acrylic acid) was studied as an unspecific reference under the same conditions. The results of selected calorimetric profiles obtained for a mixture of 90% Portland cement and 10% calcium sulfate hemihydrate are shown in Figure 4.

Without added peptide or polymer (dashed lines in Figure 4), the measured heat flow indicates rapid dissolution of hemihydrate and almost instant gypsum precipitation (initial setting, exothermic signal near t = 0), followed by a second broad feature between 2 and >30 h that corresponds to the silicate reaction (i.e., alite hydration and C-S-H formation, causing the final hardening of the concrete) and is mostly separated from the first peak of the sulfate reaction. In the presence of increasing amounts of PAA, a polymer known to interact strongly with dissolved calcium ions [25] and different solid calcium-containing minerals, [26] the reaction of both the sulfate and silicate components is significantly delayed (indicated by black arrows in Figure 4a), demonstrating that the polycarboxylate acts as an unspecific retarder during the hydration of Portland cement. This is completely different in the case of the two peptides, which progressively inhibit the crystallization of gypsum (see Figure S5, Supporting Information) and application-relevant conditions. To this end, the hydration of cement with different clinker compositions was monitored in situ using heat-flow calorimetry, in the presence and absence of the full-length peptide DYHDPSLPTLRK and the replicated local motif (DYH)_4. For comparison, neat poly(acrylic acid) was studied as an unspecific reference under the same conditions. The results of selected calorimetric profiles obtained for a mixture of 90% Portland cement and 10% calcium sulfate hemihydrate are shown in Figure 4.

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of ettringite from tricalcium aluminate and calcium sulfate hemihydrate (see Figure S6, Supporting Information).

Binding of the dodecapeptide (DYH)₄ to gypsum particles during (and/or after) their nucleation and growth in the course of CaSO₄·0.5 H₂O hydration can be independently confirmed by confocal fluorescence microscopy studies, for which the peptide was post-labeled with a fluorescent dye that reacts selectively with its primary amine groups. Corresponding images (Figure 5) show more or less even distribution of (DYH)₄ across the prominent surfaces of the formed crystals and partial incorporation of the oligopeptide into the bulk volume of the particles.

In the final part of our work, various attempts were made to translate the information gained by phage display screening from the level of peptides to synthetic polymers, which may be produced commercially at a larger scale. First, a global averaging approach was chosen, where all amino acids present in the selected library after the third panning round were analyzed and categorized with respect to their functionalities into different classes (e.g., anionic, cationic, hydrophobic, hydrogen-bonding, etc.). Based on the abundance of each category, vinyl monomers carrying similar types of functional groups were combined by random copolymerization. While this approach gave copolymers with a strong affinity to C-S-H particles with the help of corresponding PD data, it completely failed in the case of gypsum – most likely due to more pronounced selectivity, that is, a steep free energy landscape of peptide interactions with its surfaces (as evident from the strict selection of DYHDPSLPTLRK in the screening assays, cf. Figure 1). Therefore, the second concept for translation was focused on emulating the DYH motif, again using random copolymers of suitable vinyl monomers. Specifically, we chose acrylic acid...
(AA, or sodium acrylate) as a “proxy” for Asp and vinylimidazole (VI) for His, while Tyr was represented either by hydroxyethyl methacrylate (HEMA) or 4-vinylphenol (VP). These three monomers were combined in varying ratios and copolymerized under different conditions, in order to find optimum parameters for the translation of the peptide motif as judged by adsorption experiments on gypsum. Selected results of this approach are compiled in Figure 6.

The data clearly shows that random copolymers consisting of (close to) equal amounts of AA, VP, and VI (Polymers 4 and 5 in Figure 6) can compete with (DYH)$_4$ in terms of their affinity to bind to gypsum surfaces (bars in Figure 6) and, more importantly, exhibit even higher selectivity regarding their preference of gypsum over C-S-H (symbols in Figure 6). Larger deviations in terpolymer composition lead to a decrease in both adsorption on gypsum and selectivity (Polymer 3), while much weaker binding and no significant selectivity is observed when other monomers are used (e.g., HEMA as serine mimic instead of VP as tyrosine mimic, Polymers 1 and 2). These findings confirm the successful translation of the peptide motif DYH into synthetic copolymers by mimicking the functional groups of the constituent amino acids with vinyl monomer analogs, as illustrated in Figure 7.

The success of this, admittedly simplistic, concept appears all the more surprising in view of the fact that the aliphatic backbone of the synthesized terpolymers is fundamentally dissimilar to the peptide chain in (DYH)$_4$, let alone other differences such as the chemical identities of His and VI, the molecular weight of the additives, or the absence of any defined sequence of monomers in the random copolymers. Nonetheless, both the oligopeptide and the derived copolymer analogs represent two of the very few additive classes with selective effects during adsorption on gypsum and crystallization of calcium sulfate phases in reactive systems.[29]

3. Conclusion

In the present work, we have used phage display screening to identify chemical motifs maintaining strong and selective interactions with the surfaces of crystalline gypsum, an important mineral in the construction sector and other industries. The results have shown that a specific triplet of amino acids, DYH, is key to high affinity toward the inorganic substrate. Peptides derived in this way not only bind strongly to existing gypsum particles, but also proved to provide selective control over calcium sulfate hydration in complex reactive multicomponent systems such as cement (although more comprehensive cross-affinity studies will have to be performed to assess the full potential of these peptides for selective crystallization control in general). Last but not least, the straightforward translation of the DYH motif into synthetic structures by using analogous vinyl monomers yielded polymeric additives with similar selective effects. These findings highlight the potential of phage display screening (and other evolutionary selection techniques) as a rational approach to the design of efficient...
additives for crystallization control, which may replace traditional empirical product development across diverse fields of application in the future.

4. Experimental Section

**Used Materials:** All phage display screenings were performed using a Ph.D.-12 Phage Display Peptide Library Kit provided by New England BioLabs (Woburn, MA, USA), which comprises a combinatorial library of $≈10^{12}$ random 12-mer peptides fused to M13 bacteriophages via the N-terminus of their coat protein pIII. The target material for the selection process and subsequent peptide and polymer adsorption experiments was gypsum powder purchased from Sigma-Aldrich (CaSO$_4$·2H$_2$O, >99%), which consisted of well-developed gypsum crystals with a median volume-related size of ca. 500 µm (d(50) as determined by Fraunhofer diffraction) and a specific area of $\text{S}_{\text{BET}}$ (Gypsum) = 19.5 m$^2$ g$^{-1}$ (as measured by nitrogen sorption).

For adsorption experiments, C-S-H particles with a Ca/Si molar ratio of 1.50 were synthesized according to a procedure described in detail elsewhere.[22] In brief, 21.0 g calcium oxide and 15.0 g silicon dioxide were mixed with 965.0 g deionized water and stirred for four days under a protective nitrogen atmosphere. Subsequently, the formed material was separated by filtration and washed successively with pure water, water/ethanol (50/50 v/v), and pure ethanol. After drying in a vacuum, the C-S-H particles had a specific area of $\text{S}_{\text{BET}}$ (C-S-H) = 150 m$^2$ g$^{-1}$ and were stored under nitrogen. For heat-flow calorimetry, the following binders were used: Portland cement (Type 42.5 R, Schwenk Zement GmbH & Co. KG, Bernburg, Germany), tricalcium aluminate (C3A, Mineral Research Processing, Meyzieu, France), and calcium sulfate hemihydrate (Gessi, Roccastrada, Italy). Oligopeptides with different amino acid sequences were purchased from Selleck Chemicals (Houston, TX, USA) in purity of >70%, while commercial poly(acrylic acid) was supplied by BASF (Sokalan PA 20, M$_\text{n} = 2500$ Da according to gel permeation chromatography). All solutions and dilutions were prepared using water taken from a Milli-Q Direct 8 Water Purification System.

**Phage Display Screening:** For the selection phase, the following procedure was used: 10 µL of the original phage library solution was mixed with 990 µL saturated gypsum solution (to prevent the dissolution of the gypsum particles during the experiment) in standard Eppendorf vials. To this dilution, solid gypsum powder was added at a ratio of 0.1% (w/v) and the resulting suspension was incubated at room temperature for 30 min while shaking at 800 rpm. Afterward, the solid particles carrying the strongly adhering phages were separated by centrifugation for 30 s at 8000 rpm, while the non- and weakly bound phages remained in the supernatant, which was removed. The remaining solid material was washed ten times with 1 mL saturated gypsum solution by vortexing for 15 s and subsequent centrifugation, in order to further separate weakly from strongly binding phages. During the washing sequence, the Eppendorf vial was replaced three times to eliminate any potential influence of phages sticking to the polypolyethylene of the vials (i.e., to increase the selection pressure and remove peptide sequences with enhanced affinity for the vial surfaces prior to analysis). To isolate the strongly bound phages in the last step, the washed gypsum particles were dissolved in a mixture of 1 mL Tris/HCl and 100 µL Tris-buffered saline. Subsequently, the released phages were amplified in a 20 mL lysogeny broth (LB) medium containing 400 µL Escherichia Coli solution (strain ER2738, OD$_{600}$ = 0.5). After 4-5 hours of vigorous shaking at 37 °C, the phages were reprocessed according to the protocol of the supplier to prepare the selected library for another panning round. Overall, three panning rounds were performed. To increase the selection pressure along subsequent pannings, the number of washing steps was increased from 10 to 15 and finally 20. After each panning round, the selected and amplified phage library was investigated by next-generation sequencing (NGS) using the Inview Amplicon Ultra-Deep technique,[23] which provides information on the composition of the fused dodecapeptides on the phages and analyzes more than a billion sequences per panning round. All NGS analyses were performed by GATC Biotech AG (Konstanz, Germany) using a HiSeq2500 Genome Sequencer from Illumina (San Diego, CA, USA).

**Atomic Force Microscopy (AFM):** In order to visualize the binding of the selected phages on the calcium sulfate surfaces, an amplified library obtained after the third panning round (ca. 10$^{13}$ pfu mL$^{-1}$) was diluted with saturated gypsum solution by a factor of 1:100 (v/v) and incubated with solid gypsum particles (0.1% w/v) for several minutes. Subsequently, the particles were briefly rinsed with water and dried in a vacuum. AFM analyses were performed on a Bruker Dimension Icon instrument using Olympus OMCL-240-TS cantilevers. Images were taken by peak-force quantitative nanomechanical mapping (PF-QNMM) over an area of 1 µm × 1 µm in tip retrace mode, using a peak force setpoint and frequency of 5 nN and 2 kHz, respectively. The contrast was optimized by overlaying the height and adhesion channels.

**Adsorption Measurements:** The affinity of selected oligopeptides and synthesized copolymers to bind to the surface of gypsum and C-S-H particles was assessed in batch assays based on total organic carbon (TOC) analysis. First, the peptide or polymer was dissolved at a concentration of 1 g L$^{-1}$ in deionized water and the pH was adjusted to either 7 (for adsorption on gypsum) or 12 (for adsorption on C-S-H and gypsum) using aliquots of NaOH or HCl. Then, the TOC value of the solution was measured as a reference by combustion catalytic oxidation using a TOC-L instrument from Shimadzu (Kyoto, Japan). Subsequently, the respective inorganic particles were added at a solid content of 250 g L$^{-1}$ (gypsum) or 10 g L$^{-1}$ (C-S-H). After incubation for ca. 12 h, solid matter was removed by filtration and centrifugation, followed by TOC analysis of the supernatant solution. From the difference in the TOC values before and after interaction with the inorganic particles, the amount of adsorbed peptide/polymer (in mg g$^{-1}$ or mg m$^{-2}$) can be calculated. All adsorption experiments were performed at least in triplicate at a constant temperature of 23 °C.

**Circular Dichroism (CD) Spectroscopy:** To study differences in oligopeptide conformation with increasing numbers of repeat units, 100 ppm solutions of (DYH)$_4$, (DYH)$_5$, (DYH)$_6$, and (DYH)$_8$ were prepared in deionized water at pH 7 and analyzed by CD spectroscopy using a J-815 CD Spectrometer from JASCO (Pfungstadt, Germany) in a quartz cell with a path length of 0.1 cm at a constant temperature of 20 °C. CD spectra were acquired for wavelengths ranging from 190 to 260 nm at a step width of 0.1 nm and a scanning speed of 100 nm s$^{-1}$, accumulating ten successive scans per sample.

**Heat-Flow Calorimetry:** The hydration of different binder compositions (pure hemihydrate (HH)), 90% Portland cement + 10% HH or 90% C3A + 10% HH) in the presence and absence of selected soluble additives was monitored by heat-flow calorimetry using a TAM Air system from TA Instruments instrument (New Castle, DE, USA) at 20 °C. For this purpose, 3 g of the respective binder was mixed with 1.5 g (Portland cement/HH) or 3 g (pure HH, C3A/HH) water or a solution of the additive at a varying concentration (0.05–0.50%), either at native pH or after adjusting the pH to 12.0 using NaOH.

**Confocal Fluorescence Microscopy:** To visualize the presence of (DYH)$_4$ peptide on (or in) calcium sulfate particles, a 100 ppm solution of Chromo PS43 dye was first saturated with respect to gypsum and then added to solid dihydrate formed by hydration of HH under the influence of 2 ppm peptide. The fluorescence of the dye was activated upon reaction with primary amines as present in the structure of (DYH)$_4$, thus enabling selective labeling of the peptide under the given conditions. After this staining procedure, single x-y-sections of the solid particles were imaged by excitation of the dye with laser light at 488 nm, using an SP8 confocal fluorescence microscope from Leica (Wetzlar, Germany) with a water-immersion objective lens (63×, 1.2 NA). In parallel, the backscattered signal from the crystalline structures was recorded.

**Polymer Synthesis:** Free radical polymerization reactions were carried out in a semi-continuous process at 75 °C using 10 wt.% 2,2’-azobis(2-methylbutyronitrile) (Wako V59) as initiator. First, 4-vinylphenol (VP; 10 wt.% solution in propylene glycol, Sigma-Aldrich; polymers 3–5), or hydroxethyl methacrylate (HEMA; polymers 1–2), and a initiator at a varying concentration (0.05–0.50%), either at native pH or after adjusting the pH to 12.0 using NaOH.
acrylic acid (AA; polymers 1–3 and 5), or sodium acrylate (polymer 4), with vinylimidazole (VI) in isopropanol/water (50/50 w/w) were added to the reactor over a period of 3 h. Then, the initiator was added over 6 h and the polymerization continued for another 10 h. The product was neutralized and subjected to water steam distillation. Subsequently, the formed copolymer was purified by dialysis (Spectra/Por dialysis membrane; molecular weight cut-off: 12000–14000 Da), and a yellow powder was obtained after freeze-drying.

Molecular Dynamics (MD) Simulations: The mechanisms underlying the adsorption of the DYH motif on different faces of crystalline gypsum were explored by MD simulations, considering a slab of gypsum embedded in an aqueous phase containing the peptide structure. The entire simulation box was subjected to 3D periodic boundary conditions. The aqueous phase was ca. 6 nm thick, so that bulk behavior of water can be expected in its center. The four main faces of common gypsum crystals (i.e., \(\{120\}, \{010\}, \{-111\},\) and \(\{011\}\)) were prepared (see Table S1, Supporting Information) using a structural model reported in the literature.\[30\] To avoid possible detachment of calcium and sulfate ions from the surface, Ca and S atoms were restrained to their original positions from the surface, (MA)2DYH(MA)2. The rationale behind the addition of MA was to reduce the otherwise too high background solubility of the DYH unit (caused by its charged N- and C-termini). All MD simulations were performed using the LAMMPS software\[31\] with the COMPASS force field\[2\] and an integration time step of 1 fs. Simulations were run in a canonical ensemble, where the amount of substance (\(N\)), volume (\(V\)), and temperature (\(T\)) were conserved (NVT ensemble). The temperature was kept constant through a Langevin thermostat, which exchanged energy with the bath at a given frequency. The reaction coordinate was defined using two collective coordinates. The first and obvious collective variable was the coordination number of water molecules outside the slab to Ca\(^{2+}\) exposed at the surface. This variable was necessary due to the slow exchange of water in the hydration shells of calcium ions. To further accelerate convergence, the explored real-space volume was delimited by a circle with a radius of 1 nm, which restrained diffusion of the peptide model structure to a cylinder of 3 nm in height and 2 nm in diameter.

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.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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