

A Molecular-Scale Understanding of Misorientation Toughening in Corals and Seashells

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Biominerals are organic–mineral composites formed by living organisms. They are the hardest and toughest tissues in those organisms, are often polycrystalline, and their mesostructure (which includes nano- and micro-scale crystallite size, shape, arrangement, and orientation) can vary dramatically. Marine biominerals may be aragonite, vaterite, or calcite, all calcium carbonate (CaCO₃) polymorphs, differing in crystal structure. Unexpectedly, diverse CaCO₃ biominerals such as coral skeletons and nacre share a similar characteristic: Adjacent crystals are slightly misoriented. This observation is documented quantitatively at the micro- and nanoscales, using polarization-dependent imaging contrast mapping (PIC mapping), and the slight misorientations are consistently between 1° and 40°. Nanoindentation shows that both polycrystalline biominerals and abiotic synthetic spherulites are tougher than single-crystalline geologic aragonite. Molecular dynamics (MD) simulations of bicrystals at the molecular scale reveal that aragonite, vaterite, and calcite exhibit toughness maxima when the bicrystals are misoriented by 10°, 20°, and 30°, respectively, demonstrating that slight misorientation alone can increase fracture toughness. Slight-misorientation-toughening can be harnessed for synthesis of bioinspired materials that only require one material, are not limited to specific top-down architecture, and are easily achieved by self-assembly of organic molecules (e.g., aspirin, chocolate), polymers, metals, and ceramics well beyond biominerals.


1. Introduction

Biominerals are crystalline composites formed by living organisms,^[1–3] providing a source of inspiration for advanced materials synthesis.^[4–6] Different animals, such as mollusks, corals, and ascidian tunicates diverged from one another 600–750 million years ago (Ma).^[7–9] But the oldest fossils of biominerals for these three phyla are 535, 510, and 270 Ma, respectively.^[8,10] Thus, they started making biominerals independently but, surprisingly, they did so similarly: By attachment of amorphous calcium carbonate precursor particles.^[9,11] Amorphous precursors were also observed in a variety of other phyla.^[6]

The structure of biominerals has not yet been directly compared across these phyla. Thus, it is possible that structural characteristics are common to all three. If this is true, there may be a materials property that makes the selection of the shared structure beneficial to the animal. Alternatively, the shared structure may be an unavoidable and therefore uninteresting consequence of crystal growth, which is

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not functionally useful to the forming organism, as proposed by other authors.^[12,13] It is therefore worth putting these alternative and contrasting possibilities through rigorous tests.

Previous work using electron backscatter diffraction (EBSD),^[14,15] X-ray diffraction,^[16,17] and polarization-dependent imaging contrast (PIC) mapping^[18] showed that many biominerals exhibit a complex arrangement of crystal units termed crystallites, 10 nm to 10 μm in size,^[19] with diverse shapes, spatial arrangements, and crystal misorientations with respect to one another. These features are collectively defined here as mesoscale (10 nm to 10 μm) structure, or simply “meso-structure”.^[20] Each biomineral has a characteristic meso-structure, distinct from others in different animal phyla or classes.

Biominerals exhibit different structures at different scale, that is, they are hierarchical materials.^[5,21] They are frequently tougher than their components, due to the presence of organics and specific nano-, micro-, milli-, and centimeter structures formed under biological control.^[22–25] One of the most iconic and most studied biominerals is nacre, or mother-of-pearl, the iridescent inner lining of many mollusk shells.^[15,16,25–35] Nacre is much tougher than single-crystalline aragonite, with different values for fracture toughness reported at different length scales.^[36–38] Currey pioneered this fertile field, and measured the so-called work of fracture (measured as the area under the stress–strain curve, not as fracture toughness) on macroscopic samples using tensile and three-point bending tests to be 3000 \times more fracture tough than aragonite alone.^[36] Subsequent work, albeit pointing out the limitations of Currey’s tests and proposing other, better tests to measure fracture toughness, found unanimously that Currey was right, nacre’s toughness is much greater than monolithic aragonite. The quantification of the increase, however, depends on many parameters, including the nacre sample, hydration, experimental tests, fracture mechanics approach, length scales, etc... For example, Sarikaya et al.^[37] using linear elastic fracture mechanics reported an 8 \times increase in toughness. Barthelat et al. found a 30 \times increase, using the J-integral approach at the millimeter scale and hydrated samples.^[38] Using nanoindentation-based assessment of fracture toughness, instead, the reported increase in indentation fracture toughness or damage tolerance of nacre is approximately 2 \times or 3 \times greater than monolithic aragonite. This is still a significant increase, but smaller than found at greater scales, where multiple toughening mechanisms and other factors contribute to nacre toughness.^[31,33,39]

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Because they are scale-dependent, toughness values measured at different scales should not be compared directly. However, in relative terms, if a polycrystalline material exhibits microscale toughness greater than monolithic crystals, the same trend is usually observed and in fact reinforced at greater length scales. A similar trend is observed in other biological materials considered exceptional for their fracture toughness, such as the amorphous silica spicules made by sponges. The indentation-based fracture toughness of the spicules, which have a laminate architecture, is less than 2.5 \times greater than fused quartz or monolithic spicules.^[40] Nevertheless, such microscale increase in material toughness is sufficient to build huge glass spicules at the macroscale, up to 3 m in length and 1 cm in diameter, that anchor the sponge and provide high bending strength.

Here we analyzed several different biomineral mesostructures and synthetic spherulites at multiple scales with a variety of methods. Unprecedentedly, we analyzed them all quantitatively to explore the misorientation of adjacent crystallite pairs at the microscale. For this analysis, we used PIC mapping, which combines surface sensitivity (3–5 nm^[41]), spatial resolution (20 nm^[18,42,43]), and large-area imaging (10–100 μm ^[35,44]) and is therefore ideal to include many crystallites in each mesostructure. PIC mapping is not sensitive to *a*- or *b*-, only to *c*-axis orientation, as X-ray absorption spectra vary when rotating the linear polarization of the illuminating soft-X-rays with respect to the *c*-axis, but not in the *ab*-plane.^[18] Using PIC mapping, we identified a structural feature common to all biominerals analyzed here. We then used a combination of nanoindentation and scanning electron microscopy (SEM) to measure indentation-based toughness at the microscale, and molecular dynamics (MD) simulations to understand the materialsproperty correlated with this common structure at the nanoscale. Specifically, we measured fracture toughness in biogenic, geologic, and synthetic minerals, then, we simulated bicrystals containing a grain boundary, to explore the molecular-scale mechanism that made this structure common to several diverse biominerals.

2. Results

2.1. Slight Misorientation of Adjacent Crystals in Diverse Biominerals and Synthetic Spherulites Quantitatively Measured by Polarization-Dependent Imaging Contrast Mapping

The skeletons of stony corals, the spicules of tunicate ascidians, and the prisms of bivalve mollusk shells provide structural support or shelter to the animal that builds them. These three biominerals are made of one of the three calcium carbonate (CaCO₃) polymorphs: aragonite, vaterite, or calcite, respectively. Along with nacre in modern and fossil shells, including sheet and columnar nacre, six mesostructures of representative biominerals from these diverse animals are presented in the PIC maps of **Figure 1**, which were acquired using synchrotron photoemission electron microscopy (PEEM).^[45]

In these PIC maps, the color of each nanoscale pixel displays the *c*-axis direction for the crystal in that pixel, so that the in-plane and off-plane angles are displayed as the color’s hue and brightness, respectively.^[18] In color bars, the crystal habit and

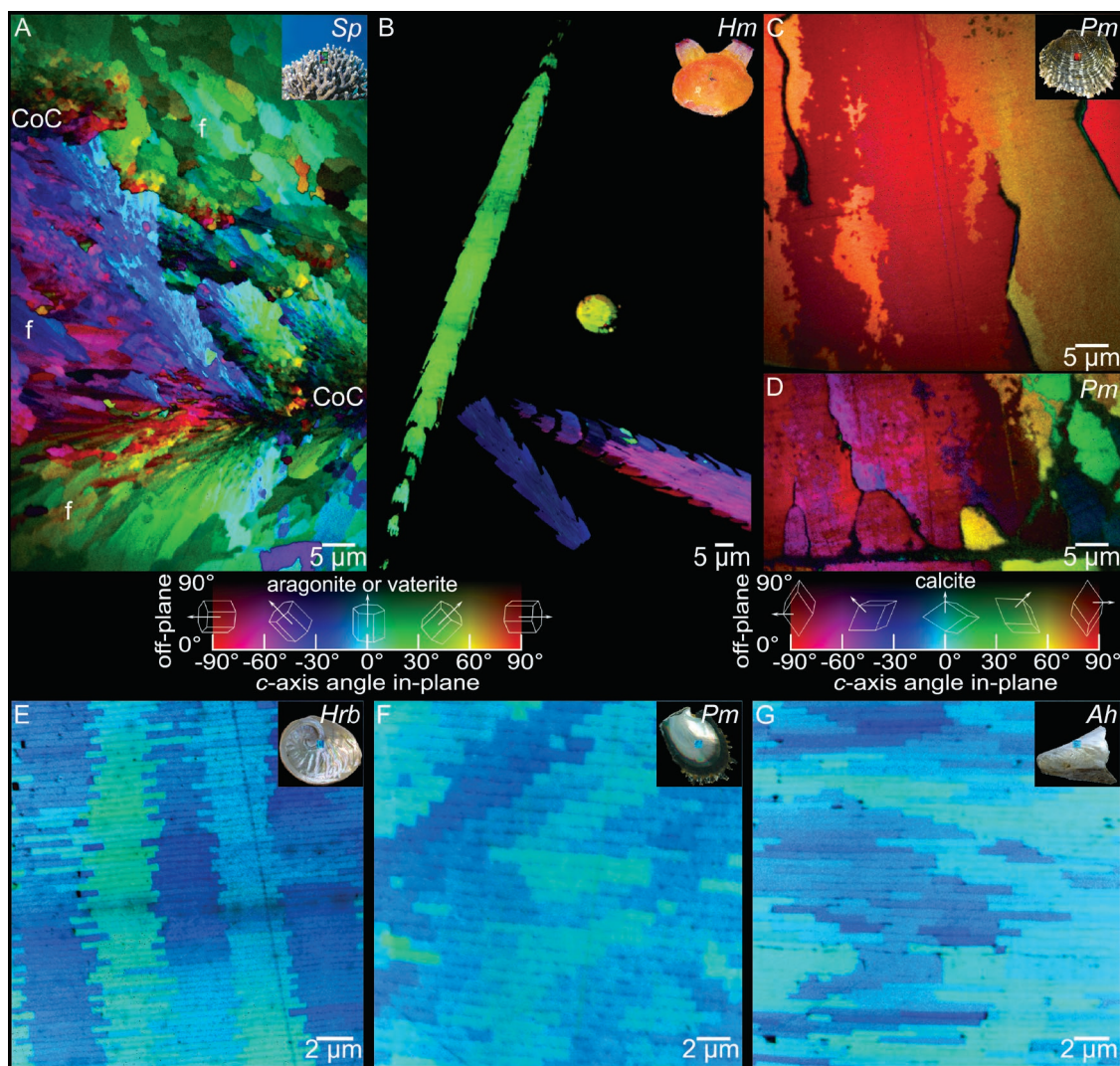


Figure 1. Polarization-dependent imaging contrast (PIC) maps display the diverse mesostructures of CaCO_3 biominerals. The orientation of the crystalline c -axis is displayed in color, including hue and brightness (see color bars, where schematics indicate crystal habits and c -axes) indicating the in-plane and off-plane angles the c -axis forms with the polarization plane. All biomineral surfaces are imaged after embedding, polishing, and coating, with the synchrotron beam illuminating at 30° grazing incidence. The approximate position of the PIC map is indicated on the photograph of each biomineral (not to scale). A) Aragonite coral skeleton from *Stylophora pistillata* (*Sp*). Centers of calcification are more nanoparticulate and extend along the non-straight line between “CoC” labels. Acicular fibers “f” form spherulites that radiate from CoCs. B) Vaterite spicules from the tunicate ascidian *Herdmania momus* (*Hm*). C, D) Calcite prisms from the Tahitian pearl oyster *Pinctada margaritifera* (*Pm*). Black pixels are organic envelopes between calcite prisms. Aragonite nacre from three diverse species. E) *Haliotis rubra* (*Hrb*) columnar nacre, characteristic of all nacre-forming gastropods and cephalopods, where cooriented aragonite tablets are stacked into columns, extending perpendicular to the nacre tablet layers. F) *P. margaritifera* (*Pm*) sheet nacre, characteristic of all nacre-forming bivalves, where stacks of cooriented aragonite tablets are staggered diagonally. G) Fossil nacre from a Miocene (13-million-year-old) *Atrina harrisii* (*Ah*). The almost perfectly preserved nacre from this fossil shell is still aragonite and shows diagonally staggered cooriented nacre tablets. Near the bottom of the image, 3–4 nacre layers are fused together, due to diagenesis. All six biomineral mesostructures show at a glance that adjacent crystallites have slight c -axis misorientations, as demonstrated by their similar colors in PIC maps. Figure 3 displays these slight misorientations quantitatively.

the c -axis direction of each mineral are also shown for additional clarity. The crystal habit is a pseudo-hexagonal prism with the c -axis along the prism axis for aragonite or vaterite, or a rhombohedron for calcite, where the c -axis comes out of an obtuse corner of the rhombohedron. Color represents the quantitatively measured orientation of the c -axis in 3D, which is displayed in 2D images.

Biomineral aragonite can be spherulitic as in coral skeletons (Figure 1A), or lamellar as found in nacre (Figure 1E–G).

Nacre is formed by 3 classes of mollusks: Gastropods, cephalopods, and bivalves. In gastropod and cephalopod nacre, cooriented tablets are organized into straight vertical columns, perpendicular to tablet layers (columnar nacre, Figure 1E), whereas in bivalves cooriented tablets are arranged in diagonally staggered stacks, which may or may not be recognizable in PIC maps depending on how the nacre cutting and polishing plane is oriented compared with the staggering direction (sheet nacre, Figures 1F, G). The structural difference between

sheet and columnar nacre is shown in Figure 1E,F. In both coral spherulite trabeculae and mollusk nacre tablets, adjacent crystals are similarly oriented, as shown by similar colors,^[46] adjacent on the color bar. Fossil nacre retains this crystallographic arrangement (Figure 1G).

Biomineral vaterite crystals in spicules formed by tunicate ascidians^[47] show a similar result: The left spicule in Figure 1B is mostly green with a few cyan pixels on the lower side of the spicule, the central one in transverse cross-section is green and yellow, the right one is magenta, with spines that are red on one side or blue on the other side, the bottom spicule is mostly dark blue, with dark cyan and dark magenta spines on either side. All of these are adjacent colors on the color bars.

Biomineral calcite in the prismatic outer layer of pinnid bivalve shells is single-crystal-like in each prism of, for example, *Atrina*^[48] or *Pinna*,^[13] whereas the pterioid bivalve shells such as *Pinctada fucata* and *Pinctada margaritifera* have polycrystalline calcite prisms.^[3,17,18,49] The *P. margaritifera* prisms in Figure 1C show the most-commonly observed case with slightly misoriented crystals, all within the red-orange range of colors. Even in the rare prisms with a greater diversity of orientations, such as those in Figure 1D, adjacent crystals have adjacent colors. See also Figures S1–S3, Supporting Information, highlighting adjacent crystals. All crystals in each image were measured, but Figure S3, Supporting Information, shows only a few pairs of adjacent crystals for clarity. A total of 832 pairs of adjacent regions in all samples were analyzed to measure their misorientations.

In all biominerals analyzed here, a key common observation is the slight misorientation of adjacent crystallites in each mesostructure. This slight misorientation is qualitatively visible at a glance in Figure 1: Adjacent crystals always have similar colors. Misorientation was observed before in various biominerals.^[3,13–18,24–26,28–30,32–34,42,44,49–57] The nanoscale misorientation of pairs of adjacent crystals, however, was never measured directly and quantitatively, nor compared across diverse biominerals as done here. Note that coral skeletons are formed by so-called plumose spherulites, that is, radial distribution of acicular aragonite crystals starting from a line and radiating in

three dimensions as the fibers in a feather duster or a bottle brush.^[42,44] In corals, each of these plumose spherulites is termed “trabecula” and the center line is called centers of calcification, labeled in Figure 1A. Across the boundary between any two trabeculae the orientation is random, thus, abrupt color changes are indeed observed in coral skeletons. These were not included in the misorientation analysis below.

Synthetic aragonite spherical spherulites were also analyzed with PIC mapping and revealed a mesostructure similar to the mesostructure observed within each spherulitic trabecula in coral skeletons. Synthetic spherulites are spherical because radial crystals start from a point, not from a line. A synthetic spherical spherulite is presented in Figure 2. In all spherulites, plumose or spherical, adjacent crystals have similar orientations.

Unprecedented and strikingly similar results are presented here in the form of histograms, showing the frequency of each misorientation of the *c*-axes across the boundary between two adjacent crystallites. All misorientations were measured as described in the Supporting Information, and in Figures S1–S3, Supporting Information. In Figure 3, no histogram shows misorientations greater than 40°, in any biomineral analyzed here. We subsequently refer to the key 1°–40° misorientation range observed in the histograms of Figure 3 as “slight misorientation,” as opposed to larger angles 41° and beyond.

We provide particular attention to nacre, one of the most iconic and well-studied biominerals. It is well-established that adjacent crystalline tablets are slightly misoriented, with *c*-axis misorientation angles within 30° (±15° from the normal to nacre layers).^[32,34] These are abiotically selected by a competition for space growth model,^[15,28,52] because nacre aragonite grows slowly along the *c*-axis and faster in the *ab*-plane,^[28] which is opposite to abiotic aragonite, growing 10× faster along the *c*-axis than along the *a*-axis.^[28] Here, we confirm and further refine this observation and find that the misorientation of adjacent tablets in nacre never exceeds 26°, including sheet and columnar nacre, from modern and fossil shells (Figure 3A). This observation is consistent across 6 diverse species of nacre-forming gastropods and bivalves in Figure 3A. This distinctive feature in a material known for its characteristic mechanical

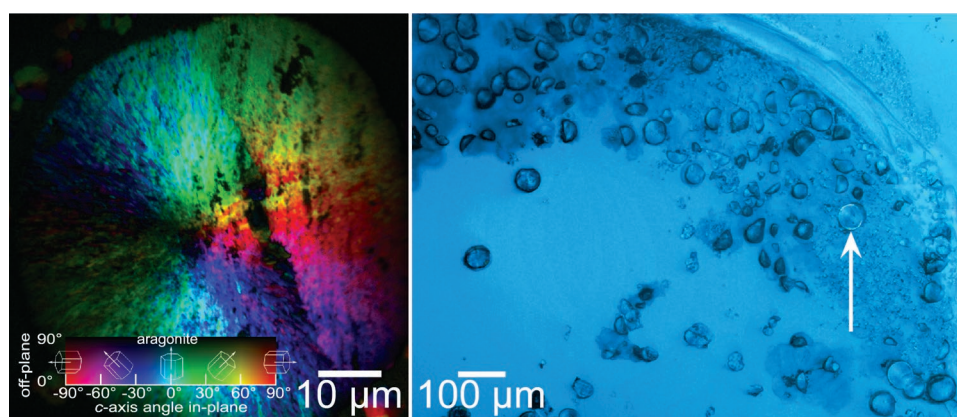


Figure 2. Slight misorientations of adjacent crystals in synthetic aragonite (CaCO_3) spherulites, lacking any organic components. A) PIC map of a spherical aragonite spherulite, grown synthetically in our laboratory. Notice that radial crystals adjacent in space have adjacent colors on the color bar, that is, they are slightly misoriented. B) Polarized light microscopy (PLM) image of many aragonite spherulites, obtained using crossed-polarizers in the illumination and analysis channels. The arrow in (B) indicates the spherulite analyzed with PIC mapping and presented in (A). Each spherulite in (B) is radially banded, as expected in PLM with crossed-polarizers.

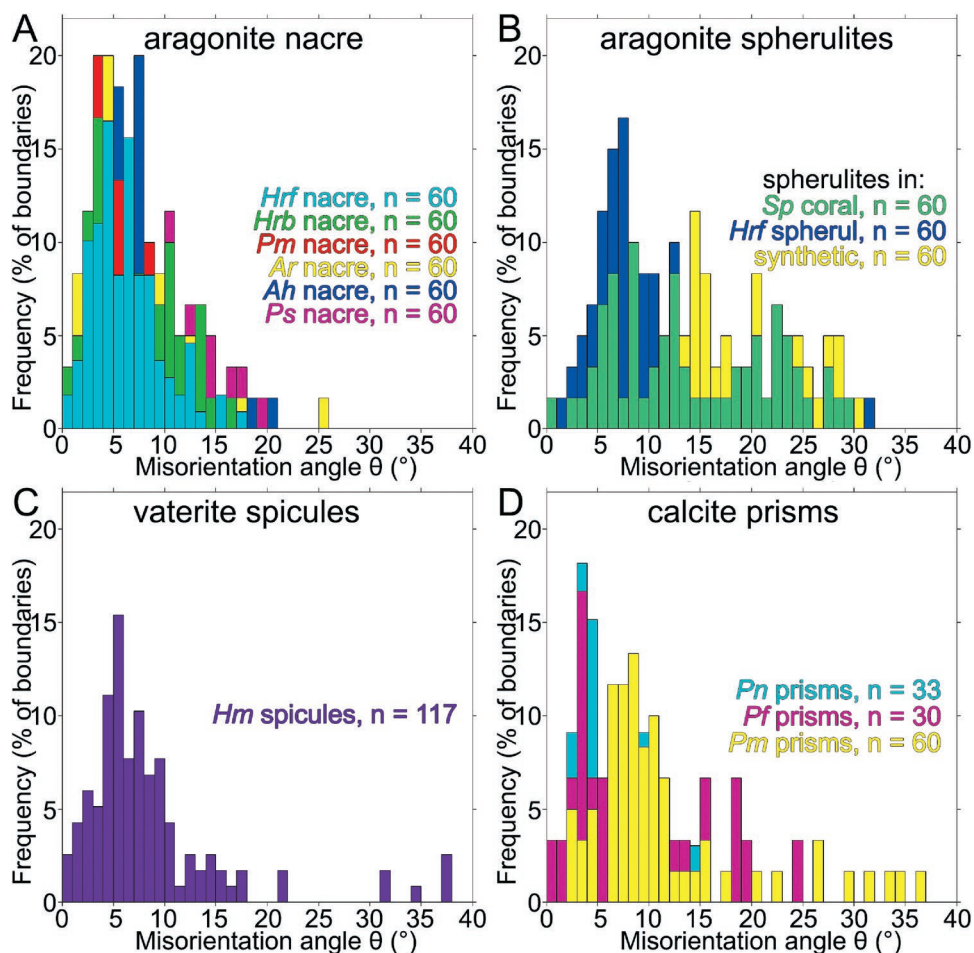


Figure 3. Slight misorientations in CaCO_3 biominerals and synthetic spherulites. A) Nacre from 6 different mollusk shells has adjacent tablets with consistently slight misorientation ($\theta < 26^\circ$), making nacre the most ordered of the biominerals studied here. The shells analyzed were from *Haliotis rufescens* (Hrf), *H. rubra* (Hrb), *Pinctadamargaritifera* (Pm), *Atrina rigida* (Ar), a 13-million-year-old *A. harrisii* (Ah), and a 66-million-year-old *Pinna* sp. (Ps). B) Biogenic and synthetic aragonite spherulites have similarly and slightly misoriented adjacent acicular crystallites ($\theta < 35^\circ$). In coral skeletons, from *S. pistillata* (Sp), only adjacent crystallites from the same trabecula were selected. Across different trabeculae misorientations were random ($0^\circ < \theta < 90^\circ$). The spherulites in Hrf are from the shell shown in Figure 4. C) Vaterite spicules from the ascidian tunicate *H. momus* (Hm). D) Calcite prisms from 3 different *Pinctada* species: *P. nigra* (Pn), *P. fucata* (Pf), and *P. margaritifera* (Pm). All these biominerals and synthetic spherulites have $\theta < 40^\circ$. Misorientation, defined as the angular distance between two *c*-axis unit vectors, was measured from pairs of adjacent regions in PIC maps as presented in Figures S1–S3, Supporting Information. Misorientation angles 41° – 90° were never found, thus, this angle range is not displayed here. In all histograms, *n* indicates the number of crystals' pairs measured. Histograms were plotted using MATLAB and overlapped using Adobe Photoshop 2023 for Mac.

toughness^[30] provided the first hint at a plausible reason for the prevalence of slight misorientation in biominerals.

Is slight misorientation useful in nacre and all other biominerals? To address this question, we looked at nacre in greater detail, and found a possible answer, after confirming previous observations. As previously observed, at the onset of nacre formation, blocky aragonite crystals seed the orientation of the first tablet crystals at the prismatic-nacre boundary. This was observed in *Pinctada fucata*,^[29] *Nautilus pompilius*,^[26] and many other mollusk shells,^[32] and in red abalone (*Haliotis rufescens*, Hrf) a layer of calcite spherulites occasionally grows within nacre, as documented by Zaremba et al.^[50] and by Su et al.^[58] We confirm that they are not calcite^[50] but aragonite.^[58] Here, we go far beyond current knowledge by observing that the acicular crystals in these spherulites directly seed the crystal orientations of Hrf nacre tablets. **Figure 4** provides direct evidence of this behavior as the growth history of nacre is recorded in

subsequent layers from bottom to top: steady-state nacre is all turquoise, including only cyan, bluish cyan, or greenish cyan in PIC maps (with *c*-axis oriented at -10° , 0° , or $+10^\circ$, respectively), as seen at the bottom and the top of Figure 4. The layer of spherulites is recognizable by the morphology of spherulites, that is, a semispherical, radial distributions of acicular crystals. Within this spherulitic layer, adjacent crystals in each spherulite are only slightly misoriented, as expected for nearby radii in a sphere, and as observed in all spherulites (geologic, synthetic, biogenic)^[42] and in all coral skeletons, including the one in Figure 1A. The spherulitic aragonite crystals directly seed nacre tablet crystal orientations, become layered like nacre, and then, as more misoriented crystals grow more slowly,^[28] faster growing crystals with *c*-axes within $\pm 10^\circ$ (turquoise hues) from the normal to the nacre layers fill space faster and thus prevail over the blue or green crystal orientations. This is evident near the center of Figure 4.

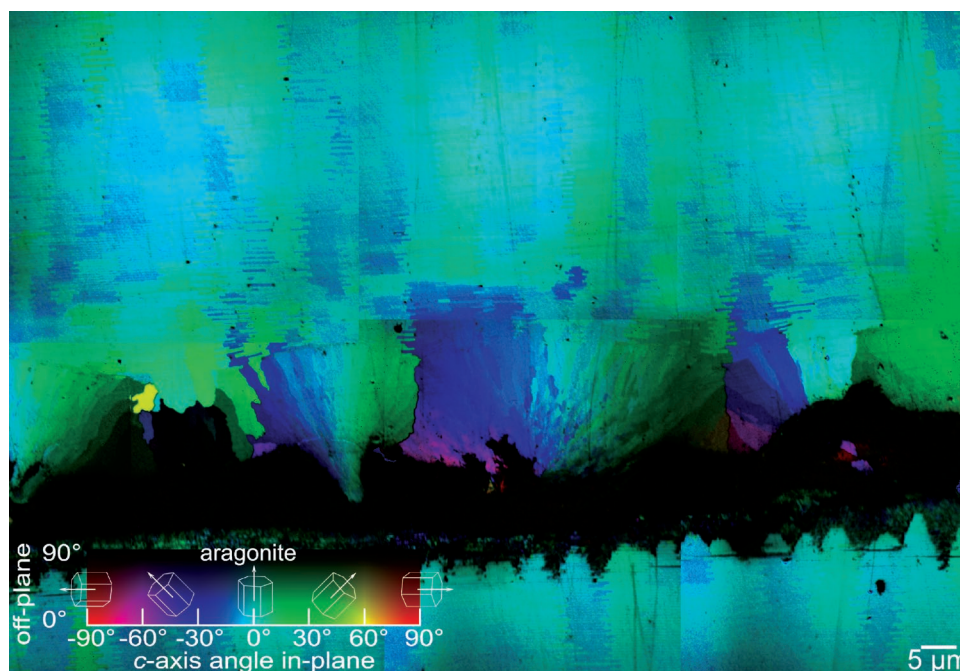


Figure 4. Slight misorientations in nacre start as spherulites. Aragonite nacre from the California red abalone *H. rufescens*. Nacre growth proceeds in the bottom-to-top direction in this cross-section. It is interrupted, an organic dark horizontal layer is formed, then spherulites nucleate, then nacre growth restarts. Notice that the *c*-axis orientations $\pm 10^\circ$ from the normal to nacre layers (including bluish-cyan, cyan, and greenish-cyan) rapidly prevail as other orientations disappear, such as green, blue, magenta, and yellow in the spherulitic layer and just above it in the first few tens of nacre layers formed epitaxially on spherulites.

Importantly, convergence to steady-state misorientations in nacre seeded by spherulites is faster than at the onset of nacre formation: at the prismatic-nacre (PN) boundary aragonite tablet orientations are random, thus, misorientations reach 90° , and slowly decay to steady state after $\approx 50 \mu\text{m}$ from the first nacre layer.^[28,32] At the spherulites–nacre (SN) boundary, instead, misorientations reach a maximum of 45° , and rapidly decay to steady state after only $\approx 20 \mu\text{m}$. Figure S4, Supporting Information, shows both results, measured in *Hrf* nacre. This observation suggests that achieving steady-state slight-misorientation faster during shell formation may be useful to the animal, and, therefore, that a materials property may confer that advantage. To explore this suggestion, we used nanoindentation to compare slightly misoriented biominerals and synthetic spherulites to single crystals.

We stress that PIC mapping is not sensitive to azimuthal rotations of the crystal around the *c*-axis, only to polar rotations of the *c*-axis. Thus, only misorientations of *c*-axes were measured and tested by nanoindentation and MD simulations. Azimuthal misorientations, which may very well play a role, remain unexplored and could be investigated in future work.

2.2. Toughness of Slightly Misoriented versus Single-Crystalline Aragonite Crystals, Measured by Nanoindentation

To investigate the relationship between slight misorientation and fracture resistance, we measured the local fracture toughness using nanoindentation. This is an established approach to characterize local fracture behavior of engineering and

biological materials. This method is used here to elucidate the origins of toughness in biominerals by comparing geologic, synthetic, and biogenic aragonites, using the same instrument, conditions, and settings. We followed a procedure widely adopted for biominerals,^[23,40,59,60] in which a sharp cube-corner diamond indenter is used to induce cracking at much lower loads than with less sharp standard Berkovich indenter tips. Indents obtained with increasing loads, from mN to N, are then observed by SEM to quantify surface damage. To compare diverse samples, the indentation fracture toughness K_{c} was calculated using the following formula:^[61]

$$K_{\text{c}} = \chi \left(\frac{E}{H} \right)^{1/2} \left(\frac{P}{c^{3/2}} \right) \quad (1)$$

where χ is a numeric parameter characteristic of the indenter tip geometry ($\chi = 0.04$ for a cube corner), E is the elastic modulus, H is the hardness (measured with nanoindentation at low load/penetration depth and presented in Figure S5, Supporting Information), P is the indentation load, and c is the crack length, measured from the center of the indent.

Single-crystalline geologic aragonite exhibits a typical brittle behavior: radial cracks are already present at lower loads (1–10 mN, Figure S6A, Supporting Information) and increasing the penetration force causes extensive crack propagation along multiple directions (Figure 5A). This behavior is consistent with the observed low fracture toughness, that is, $K_{\text{c}} \approx 0.31 \text{ MPa m}^{1/2}$ for geologic aragonite (Figure 5G).

Importantly, we chose to characterize fracture toughness with nanoindentation to probe precisely the same microscale

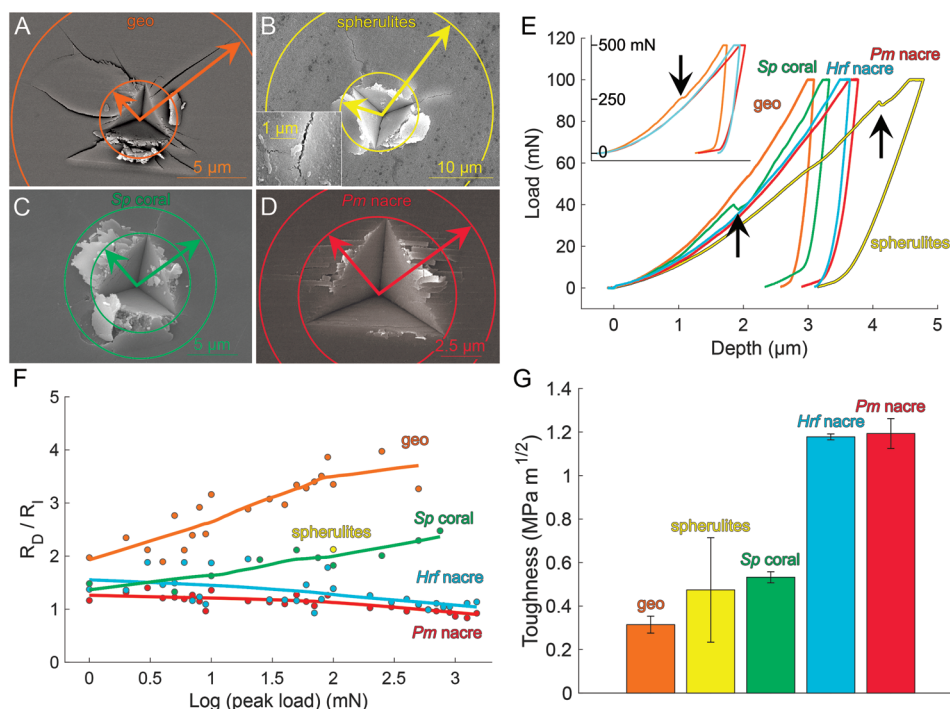


Figure 5. Indentation fracture toughness is greater in aragonite biominerals and synthetic spherulites, compared to single-crystalline geologic aragonite. A–D) SEM images post-indentation with a peak load of 100 mN. In (A), (B), and (D) the indentation direction was perpendicular to the aragonite *c*-axis, in (C) multiple crystals in coral skeleton have unknown orientations. A) Geologic aragonite crystals indented perpendicular to *c*-axis, B) synthetic spherulites (inset shows additional toughening mechanisms: crack deflection, microcracking, and crack branching), C) coral skeleton, and D) *Pm* nacre (indented perpendicular to *c*-axis, that is, with nacre tablet layers horizontal in the image). *Hrf* nacre has a behavior qualitatively similar to *Pm* nacre (Figure S6C, Supporting Information). SEM images are used to extract the radius of the residual indent R_I and of the entire damaged area R_D by fitting the smallest possible circle centered on the indent. E) Typical indentation curves at a peak load of 100 mN: synthetic and coral aragonites show characteristic pop-in events (highlighted by arrows), associated with sudden damage beneath the contact surface. Geologic aragonite also shows such discontinuities but at higher loads, *Pm* and *Hrf* nacre do not (see inset), at either lower or higher loads, indicating a progressive damage accumulation but no abrupt pop-in events. F) Ratio of mean damage radius (R_D) and indentation radius (R_I) plotted versus indentation peak load (trend lines are locally weighted scatterplot smoothing, LOWESS, regression fits with a smoothing factor of 0.8). This metric quantifies whether damage tends to localize around the indent (as in *Pm* and *Hrf* nacre) or to propagate (as in geo aragonite). Both coral and synthetic spherulites have intermediate behavior. G) Experimentally measured indentation fracture toughness (abbreviated as “Toughness”), obtained by fitting the correlation between damage radius (R_D , a measure of crack length) and indentation load (see Methods in Supporting Information, and Figure S7, Supporting Information).^[40,60] Data are presented as mean \pm SD over multiple locations within the same sample, except for the spherulites, where 4 different spherulites were measured. Comparison of all the samples demonstrates that slight misorientations, present in synthetic spherulites, coral skeleton, and nacre but absent from geologic aragonite, correspond to greater toughness.

across all the different samples, so the comparison of toughness is possible and quantitatively relevant.

Most interestingly, synthetic spherulites, which have slightly misoriented adjacent crystals but lack any organic interfaces, show a more damage tolerant response, with indents accommodated by both radial cracking and shear induced damage. A closer examination of the damaged surface reveals that the synthetic spherulites are granular, thus, additional toughening mechanisms such as tortuous crack paths, crack deflection, and branching (Figure 5B inset) may also contribute to the observed 50% greater average toughness ($K_c \approx 0.47$ MPa m^{1/2}) compared to single-crystalline geologic aragonite.

A similar fracture behavior is observed in coral skeletons: at low to moderate loads (1–25 mN) the contact stress field generates localized pile-up and chipping damage (Figure S6B, Supporting Information). Higher loads are then required to induce radial cracks (Figure 5C and Figure S6B, Supporting Information), which, however, never propagate far from the indent as suggested by the ratio of damage radius R_D to indent size R_I ,

which is smaller for coral skeleton than for geologic aragonite (Figure 5F). The corresponding average fracture toughness for coral skeleton is $K_c \approx 0.53$ MPa m^{1/2} (Figure 5G), a 70% increase with respect to geologic single-crystalline aragonite.

Nacre showed the most damage tolerant response: the damage region always remained confined near the indent, as also shown by the ratio of damage to indent radii (Figure 5F) due to multiple toughening mechanisms, including sliding of aragonite tablets, tablet pull out, crack deflection at tablet-tablet boundary in the same layer, within tablets, and at the tablet-organic sheet interface (Figure 5D and Figure S6C,D, Supporting Information). As a result of all these mechanisms combined, nacre has the highest average fracture toughness, with no difference between sheet and columnar nacre ($K_c \approx 1.19$ MPa m^{1/2} and $K_c \approx 1.18$ MPa m^{1/2}), in *Pm* and *Hrf* nacre, respectively. Nacre shows an almost 300% increase in toughness with respect to geologic aragonite.

Differences in fracture behavior are also reflected in dissimilar indentation curves (Figure 5E): geologic aragonite,

synthetic spherulites, and spherulitic coral skeleton show sudden jumps in the load versus depth curves (arrows in Figure 5E, and inset), which are hallmarks of fracture or sudden damage events beneath the contact point.^[2] Such discontinuities are not observed in nacre, even at higher loads, suggesting more gradual damage accumulation and greater damage tolerance.

Comparison of toughness values for all aragonite samples demonstrates that biominerals, including nacre and coral skeletons, are considerably tougher than single crystalline aragonite (Figure 5G). This is consistent with previous studies on geologic and biogenic crystals,^[31,33,55,62] identifying the role of organic inclusions and other mechanisms (e.g., friction between rough interfaces and sacrificial mineral bridges) as possible amplifiers for fracture toughness. It is worth mentioning that azimuthal orientation could also influence nanoindentation results. Specifically, changes in the azimuthal angles of the indenter were shown to impact the measured hardness, but not the indentation modulus in both geologic minerals and biominerals.^[63] The impact of azimuthal rotations on indentation fracture toughness is not considered here, and it could be explored in future studies. A novel aspect reported here is the fracture behavior of synthetic spherulites: Those structures were grown in the absence of anything organic, and they are on average 50% tougher than geo aragonite. Importantly, the same result is obtained also when indenting aragonite in a different direction with respect to the *c*-axis (Figure S8, Supporting Information). This observation suggests that, besides structural characteristics such as organic interfaces,^[56,64] misorientation of the minerals also contributes to the toughness of CaCO₃ biominerals.

2.3. Toughness of Aragonite, Vaterite, Calcite in Molecular Dynamics-Simulated Misoriented Bicrystals, Measured by Mode I Fracture

Indentation experiments indicate that misorientation itself contributes to material toughness, even in the absence of organic components at the interface. However, even when probing synthetic spherulites without organic components, other factors such as shear stresses and material pileup around the indenter likely influence the mechanical measurement. To isolate the effect of misorientation, we next perform MD simulations of CaCO₃ bicrystals. We subject these bicrystals to tensile fracture loading and measure toughness as a function of misorientation within each bicrystal. Specifically, the *c*-axes of the two crystals within each bicrystal are either cooriented ($\theta = 0^\circ$) or misoriented in the range of $\theta = 5^\circ$ – 90° . The right crystal is notched, and a tensile load is applied vertically to initiate mode I fracture. The crack propagates from right to left, starting from the notch and either continues straight through the material or is deflected by the bicrystal interface. The crystal size, geometry, and loading are illustrated in Figure 6A. This geometry was selected because in the case of nacre with horizontal tablet layers, if a crack propagates vertically, it quickly encounters organic sheets and is therefore deflected at those interfaces. In this study we tested crack deflection at the interface of tablets in the same nacre layer, because nacre's tensile strength in the

direction parallel to the tablet layers is far greater than in the normal direction.^[51,53] None of the other mechanisms known to toughen nacre^[54] are included in these simulations, on purpose, to isolate the specific effects of slight misorientation.

All three CaCO₃ polymorphs were investigated, and each simulation was repeated three times to account for slight stochastic differences in fracture propagation. Part of the results are presented in Figure 6B and clearly show greater crack deflection at a slight misorientation angle of 10° , compared to the single crystal (0°). Crack deflection is smaller when the misorientation is increased to a larger 45° angle. Material toughness as a function of misorientation, measured as the integral under the stress–strain curves (Figure S9, Supporting Information), peaks at misorientation angles $\theta = 10^\circ$, 20° , and 30° for aragonite, vaterite, and calcite, respectively, as shown in Figure 6C. Aragonite was additionally subjected to mode I fracture in the normal direction. These cracks simply propagate along the bicrystal interface as expected, independent of misorientation angles, as presented in Figure S10, Supporting Information.

In the case of slight misorientations, increased crack deflection across the interface correlates with increase in toughness. However, at larger angles aragonite exhibits another increase in toughness distinct from the slight misorientation effect. This is likely due to the anisotropic fracture properties of single-crystal aragonite, which has higher toughness when pulled perpendicular ($\gamma = 90^\circ$) to the *c*-axis than parallel to it ($\gamma = 0^\circ$), as shown in Figure S11B, Supporting Information. Additionally, vaterite single-crystals exhibit secondary fracture from the upper unnotched plane when *c*-axes are rotated to large angles away from the tensile direction, as shown in Figure S11C, Supporting Information, resulting in crack paths that appear tortuous independent from misorientation toughening. Similarly, calcite single-crystals fail much more readily as their *c*-axes are rotated away from the tensile direction, with lower yield strains as shown in Figure S11D, Supporting Information, acting as a source of secondary fracture, and providing more tortuous fracture paths without correspondingly high toughness. All three of these large-angle behaviors are due to fracture properties of single-crystals, not to large misorientations of bicrystals.

Despite the above, polymorph-specific, single-crystal behaviors, the common trend in Figure 6 is clear: Slight misorientation makes bicrystals tougher. Specifically, direct comparison of the MD results precisely illustrates the multiplicative effect of slight misorientation on toughness. Misoriented bicrystals of aragonite, vaterite, and calcite become 2.5, 2, and 3 times tougher, respectively, than their cooriented crystal structures. This comparison is presented in Figure S12, Supporting Information, which includes the literature result obtained for hydroxyapatite in human enamel using similar MD simulations.^[65]

3. Discussion

The comparison of biomineral mesostructures presented in Figure 1 yields surprising insights, because it reveals that, despite wildly different mesostructures, adjacent crystals have

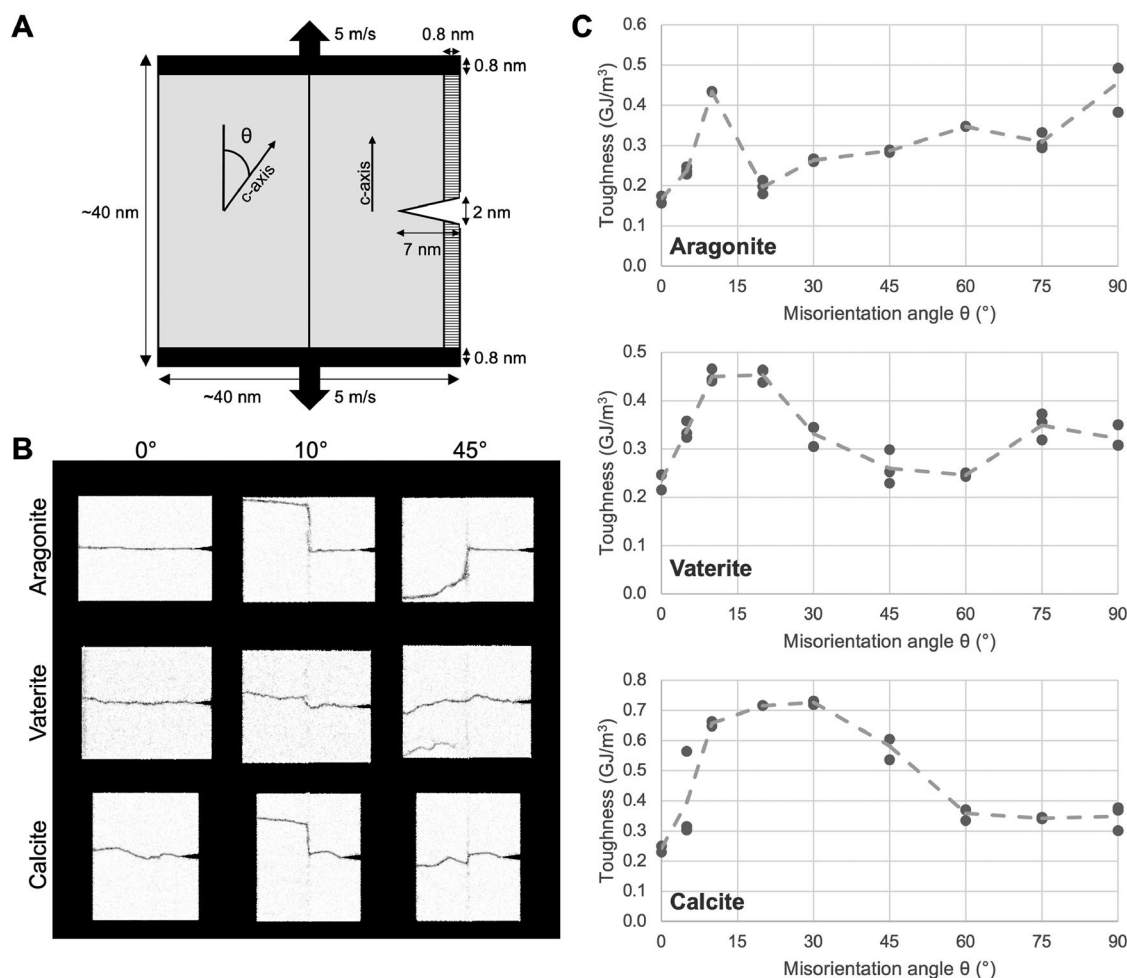


Figure 6. Slight misorientation toughens nacre, when cracks propagate through tablets parallel to nacre layers in MD simulations. A) Aragonite, vaterite, and calcite were subject to mode I fracture. Bicrystal structures were designed to resemble two adjacent nacre tablets in the same nacre layer, and the interface between them. The right crystal *c*-axis parallel to the tensile direction and vertical and the left crystal *c*-axis rotated in the plane of the figure by a misorientation angle θ . B) The introduction of a misoriented interface results in qualitative crack deflection, with a larger effect at 10° than 45°. C) Fracture toughness calculated across a range of misorientations quantitatively shows that toughness has a peak at small angles: Approximately 10°, 20°, and 30° for aragonite, vaterite, and calcite, respectively. Each simulation was repeated 3 times.

similar colors, that is, are slightly misoriented at the micro- and nanoscales.

No two biomineral mesostructures could have more diverse morphologies than nacre, with tablets arranged as brick-and-mortar, and coral skeletons, with acicular fibers arranged in spherulites. Yet, from the point of view of slight misorientation of adjacent crystals, nacre and corals skeletons are quite similar. One could even say that nacre is spherulitic. In fact, nacre can start spherulitic, as shown in Figure 4, and then slight misorientations are selected abiotically, by competition for space. If one accepts that slight misorientations are better than larger ones, then faster convergence to steady-state-nacre misorientations of 20° within 20 μm , instead of the usual 50 μm observed at the prismatic-nacre boundary,^[28,32] makes nacre tougher faster, limits the volume of lower-toughness nacre, thus, it makes the overall nacre layer and its mesolayers^[51] more durable. It is unclear at present how much a thinner greater misorientation layer makes the overall material tougher. As an aside, many blocky, equant, aragonite crystals in the

literature were incorrectly termed spherulitic by other groups and ours,^[28] because they are equant and single-crystalline, but they are not a distribution of acicular crystals, slightly and gradually changing in orientation like the spherulites in Figures 1A and 2. Spherulitic aragonite, forming after an organic layer in *Hrf* nacre,^[58] seeds the orientation of nacre tablets as shown in the PIC map of Figure 4. This map includes both spherulites and nacre tablets and shows that cyan acicular crystals in spherulites become layered nacre tablets with the same color and orientation. Thus, biominerals that may seem completely distinct under a traditional lens may share features after all.

Indeed, the observation that diverse biominerals share slight misorientations suggests that these could provide an evolutionary advantage. Nanoindentation experiments on biominerals and synthetic spherulites indicated that slight misorientation is, among other factors, a potential toughening mechanism. However, neither experiment could prove direct correlation of toughness and slight misorientation, because the systems are too complex and feature many toughening

strategies all occurring simultaneously, making it impossible to isolate misorientation for a direct comparison. Therefore, we employed MD simulations to isolate the effect of the misorientation in a controlled bicrystal system, and determine a clear relationship between slight misorientations and toughening in bicrystals. No parameters other than misorientation angles were varied, thus, the results of MD experiments are clear: slight misorientations of 10°, 20°, 30° in aragonite, vaterite, and calcite, respectively, most effectively toughen bicrystals.

Importantly, nanoindentation at the microscale and MD simulations at the nanoscale are two independent methods of obtaining toughness trends and, although these are not directly and quantitatively comparable to one another, they agree with one another about greater toughness at slight misorientation angles. The measured microscale toughness results are in good agreement with previous data at the same scale.^[31,33,39]

We stress that MD simulations were done on the simplest possible system, including only two sintered crystals and the interface between them. They do not contain any hierarchical structure that could contribute to toughness, or any additional materials at the interface, which could also toughen. These additional features, ubiquitous in natural biominerals, were omitted on purpose to test the effect of crystal misorientation alone, not in combination with other mechanisms.

Azimuthal misorientations of adjacent crystallites may also play a role in toughening biominerals, but those remain unexplored. Future studies could detect such misorientations using EBSD, accompanied by nanoindentation or MD simulations, to explore that additional effect.

In previous studies, remarkable improvements in materials properties were observed with low-angle and high-angle grain boundaries in metals and superconductors.^[66] The separation between the two regimes was always 15°.^[67] The misorientations of adjacent crystals observed in natural biominerals and synthetic spherulites do not fit squarely into either category. They are indeed observed at grain boundaries, but they are not segregated along the same low-angle (<15°) or high-angle (>15°) criteria. Instead, PIC mapping experiments confirm that the observed misorientations peak at approximately 5°–15° but they include many other angles between 1° and 40°, albeit less frequently (see Figure 3 histograms). Thus, a new category must be added: slight misorientation, 1° < θ < 40°, as presented here.

Furthermore, MD simulations were done at the nanoscale whereas misorientation and nanoindentation-toughness were measured at the microscale. Performing these measurements at the same scale is challenging at present and could not be done here.

Can an organism benefit at the macroscale from the micro- or molecular-scale toughening observed here? If the aforementioned example of sponge spicules is a guide, then the answer may be yes, and in fact the macroscale toughness may build upon the molecular-scale toughness, at the micro- and macroscales, and thus significantly increase toughness with scale.

Because increasing values of fracture toughness were measured at increasing length scales in hierarchical heterogeneous composites such as nacre (see introduction), here we chose to explore fracture toughness at the smallest possible scales: the microscale with indentation and the molecular scale using MD simulations. At the molecular scale, MD simulations reveal the

Table 1. Comparison of MD-predicted misorientation toughness maxima and observed misorientation angles.

Mineral	Maximum toughness in MD simulations	Most frequently observed misorientation angle
Aragonite	10°	5° in nacre
Aragonite	10°	5° in shell spherulites
Aragonite	10°	15° in coral skeletons
Vaterite	10°–20°	5° in tunicate spicules
Calcite	20°–30°	5°–10° in <i>Pinctada</i> prisms

direct relationships between crystal orientations and toughness to a level inaccessible by experiment alone.

Many toughening mechanisms have been observed in biological materials.^[24,68] Even slight misorientation of adjacent crystals was recently observed in human enamel and shown, with MD simulations similar to those presented here, to be functional in preventing fracture.^[65] Now the present work recontextualizes this enamel observation as a piece of a larger view that slight misorientation toughening is a more general mechanism across many biominerals. The fact that enamel is so different in mineralogy and mesostructure from CaCO₃ biominerals further supports the idea that slight misorientations toughen biominerals, and it may therefore provide evolutionary advantages to the forming organisms.

Comparing the maxima predicted by MD simulations for aragonite, vaterite, and calcite, respectively, they do not correspond precisely to the angles most frequently observed in the biogenic and synthetic systems, as shown in **Table 1**.

The values in Table 1 do not correspond precisely. For both MD-simulation and PIC mapping experiments, however, the maxima occur at angles below 30°–40°. Future work by the bio-inspired materials synthesis community will be necessary to fully harness the slight misorientation-toughening observed here, and to predict the parameters that can best control it.

4. Conclusions

We have compared biomineral structures and surprisingly found that diverse organisms make their biominerals with adjacent crystals similarly slightly misoriented. MD simulations and nanoindentation experiments concur with one another on this observation, albeit with slight numeric differences. This observation in natural biominerals suggests that synthetic materials could be made tougher by small misorientations. The latter is expected to be relatively simple, because many synthetic, geologic, or biogenic crystals spontaneously grow spherulitically,^[42,44] that is, as needle-like crystals radiating from common centers, and, in all spherulites, adjacent crystals are always slightly misoriented, and therefore tough. A key advantage of the bioinspired slight-misorientation-tough spherulitic structure is that it only requires one, not multiple materials with a specific architecture templating another in a top-down approach. It can be easily achieved by self-assembly of organic molecules such as aspirin^[69] and cocoa butter in chocolate,^[70] polymers,^[71] graphite in cast iron,^[72] semiconductors,^[73] and many more systems, well beyond calcium carbonate

biominerals. Thus, the observation of slight-misorientation-toughening in this work is relevant to stimulate the synthesis of bioinspired materials with useful functions and enhanced toughness.

5. Experimental Section

Detailed materials and methods are provided in Supporting Information.

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.

Author Contributions

P.U.P.A.G. conceived the idea that slight misorientation is similar across biominerals for a materials reason, and that MD simulations could be used to test this idea. M.J.B. and A.J.L. designed the MD simulations and fracture analyses, and A.J.L. carried out the molecular-scale simulations, collected the data, and visualized the results. The simulations were analyzed and interpreted (in conjunction with the experimental data) by A.J.L., M.J.B., and P.U.P.A.G. C.A.Sc., C.A.St., A.S., and P.U.P.A.G. acquired all the PIC mapping data. D.R. designed the nanoindentation experiments and SEM analysis. A.T., A.C., L.M., and

D.R. acquired and processed all nanoindentation data. L.M., P.C., and Y.D. performed SEM and A.T., A.C., and L.M. analyzed post-indentation images. Nanoindentation and SEM data were interpreted by A.T., A.C., L.M., D.R., and P.U.P.A.G. C.A.Sc. prepared the synthetic spherulites. C.A.St. and P.U.P.A.G. collected and processed all the misorientation data for the histograms in Figure 3. P.U.P.A.G. prepared Figures 1–4, A.T., A.C., L.M., and D.R. prepared Figure 5, A.J.L. prepared Figure 6. P.U.P.A.G. wrote the manuscript, all co-authors edited it.

Data Availability Statement

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

Keywords

crystal misorientation, nacre, nanoindentation, synthetic spherulites, toughening

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