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Biomaterial mesostructure

Pupa U.P.A. Gilbert* 

Biomaterials formed by animals are most frequently calcium carbonate or phosphate polycrystalline materials with complex hierarchical structures. This article will focus on the 10-nm–10- μm scale, termed “mesoscale,” at which the “mesostructure” differs greatly across biomaterials, is relevant to their mechanical properties, and reveals formation mechanisms in sea urchin teeth, mollusk shell prisms and nacre, human enamel, and coral skeletons. This article will conclude by focusing on important unanswered questions to inspire future research.

Introduction

Biomaterials are crystalline^{1–16} or amorphous^{17–19} minerals formed by living organisms. They are the hardest parts of every organism and have diverse functions, including skeletal support, magnetic and gravitational field sensing, accelerometry, light focusing, flotation, and ballast. Unexpected and exciting new structures, properties, and formation mechanisms are frequently discovered in natural biomaterials, such as the recent dual scale, single-crystalline calcite in a sea star revealed by Li et al.²⁰ and the latest installments of the ongoing debate on the fiendishly complicated structure of pteropod shells.^{21–28} Biomaterials also provide extremely useful ideas for bioinspired synthetic materials.^{29–41}

Aragonite and calcite (both polymorphs of CaCO_3) are the final, stable crystalline phases in many marine biomaterials. A major discovery was made by the Addadi–Weiner group, when they showed that calcite in sea urchin embryonic spicules⁴² and adult spines⁴³ does not form ion-by-ion, but through an amorphous precursor phase. Since then, multiple amorphous precursor phases have been identified in a variety of biomaterials;^{44–63} thus, formation via transient amorphous precursors is well established.

All crystalline biomaterials thus far explored appear to form by particle attachment,⁶⁴ where the attaching particles are amorphous and subsequently crystallize. Surprisingly, the

attaching particles are made of the same amorphous precursors in phylogenetically very diverse animals.⁶⁵ Attachment of amorphous particles is not only shared by modern forming biomaterials, but dates back to the earliest known animals in the Ediacaran, 550 million years ago.⁶⁶

Digging deeper into convergent biomaterialization, an integrated model for CaCO_3 biomaterialization was produced, including all current knowledge of CaCO_3 formation in different marine organisms, including seashells, other shells from foraminiferans, coccolithophorids, and brachiopods, sea urchin spines and spicules, and coral skeletons.⁶⁵

Mesostructure = size, shape, spatial arrangement, and orientation of crystallites

Despite its complex morphology shown in **Figure 1**, each sea urchin spine is a single crystal of calcite. The single crystallinity of the sea urchin spine, however, is the exception, because crystalline biomaterials most frequently are a composite of organics and minerals interspersed, where the organics may be organized in layers (e.g., sponge spicules [also in **Figure 1**] or nacre [**Figure 2**]), or more complex morphologies and the mineral is polycrystalline with many crystallites filling space (e.g., in nacre, coral skeletons, or tooth enamel). Crystallites range in size between 10 nm and 10 μm ,⁶⁷ and their shapes, spatial arrangements, and crystal orientations are diverse and

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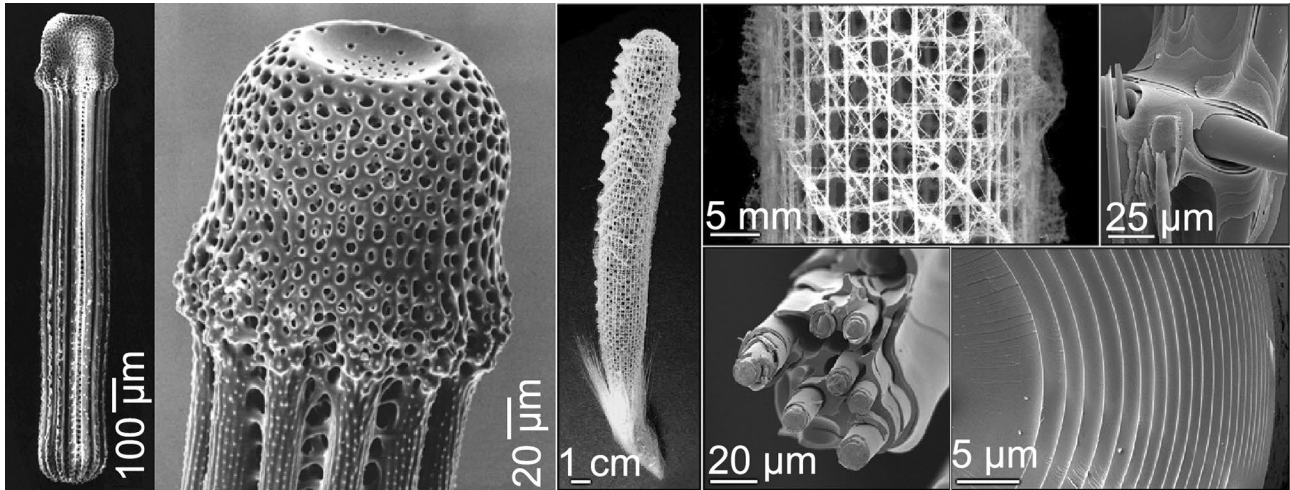


Figure 1. Scanning electron microscope image of a sea urchin spine and sponge spicules. The sea urchin spine (left two panels) diffracts like a single crystal of calcite, despite its intricate morphology very far from a calcite rhombohedron. Adapted from Reference 80. The sponge spicule (right 5 panels) is made of amorphous silica, but it is layered, which confers its resistance to fracture. Adapted from Reference 19.

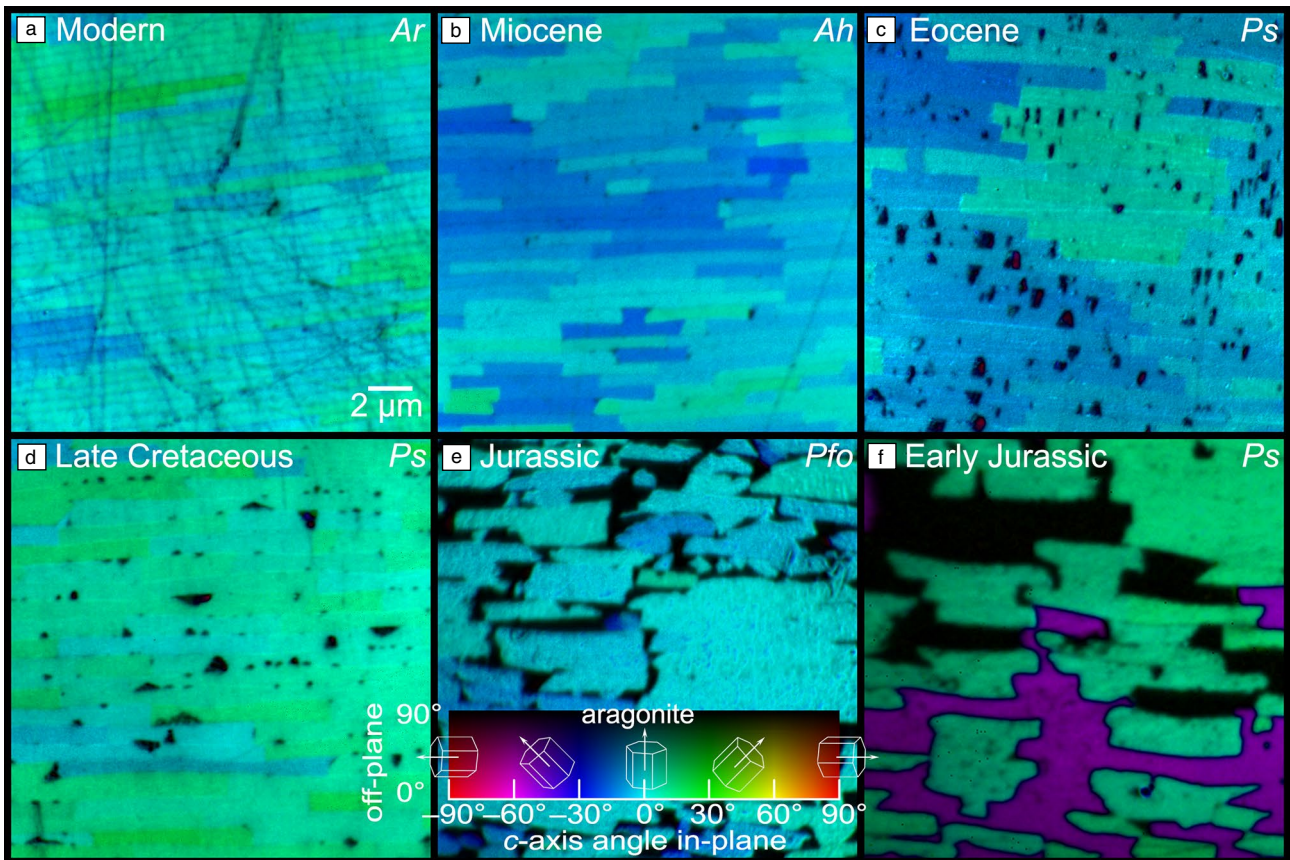


Figure 2. Nacre, or mother-of-pearl, is the iridescent inner lining of many mollusk shells. Here, only nacre from bivalves is shown in modern and fossil shells, from 0- to 200-million-years old. The ultrastructure is reasonably well preserved, but holes are more frequent in older shells (black pixels indicate no aragonite). Part of the Jurassic shell is recrystallized as calcite, and this is wildly misoriented compared to pristine nacre, as shown by the purple tablets instead of the usual turquoise colors of nacre visible in all other panels. In all of these polarization-dependent imaging contrast maps, color represents the orientation of the crystalline c-axis of aragonite or calcite crystalline, including hue and brightness indicating in-plane and off-plane orientations. The two letters on top right of each panel indicate the genus and species. Data from Reference 142.

characteristic of each biomineral. It is useful, therefore, to use a collective term for all of these characteristics: mesoscale (10 nm–10 μ m) structure, or simply “mesostructure.”

The evolution of mesostructure

Animal biominerals must be hard and stiff to perform their function, most frequently related to prey–predator mechanisms: predation weapons include teeth, fangs, and horns; prey sheltering armors include shells, carapaces, and exoskeletons.

The shape of these biominerals is finely tuned by evolution by natural selection; thus, a biomineral is not just a rock. The materials properties are also honed (pun intended) by evolution. Biominerals are invariably harder, stiffer, stronger, or tougher than their components. This is achieved in part by hierarchically structuring the biomineral, which means that the structure is biologically controlled at multiple scales at the same time.^{68–73}

Once a biomineral has been fine-tuned by evolution for a specific function, it does not stop changing. It keeps changing due to random mutation. This is fundamentally different from the way human design occurs: once we are done designing a material we stop. In nature, instead, mutation is random, and it doesn't stop, no matter how good the material already is; it keeps changing through generations. It evolves. If a mutated material happens to be a little harder, or stiffer, or tougher, then it provides a competitive advantage to the host organism and thus prevails as either a better predator or a more protected prey. The materials properties, therefore, are the result of constant change kept in check by natural selection during prey–predator encounters. The ultimate materials properties of biominerals, therefore, are a direct result of evolution.^{74–79}

How to visualize mesostructures

The most common and readily available method to visualize every aspect of biomineral mesostructure is electron backscatter diffraction (EBSD), which has been used extensively in biomineralization^{13,77,80–86} and it works perfectly, as long as the individual crystallites are big enough, greater than 200 nm. X-ray micro- and nano-diffraction work well for biominerals,^{87–98} but it is very hard to understand where, along the penetrating x-ray beam, each diffracting crystallite is located. X-ray diffraction tomography will solve this problem; therefore, it holds great promise, but thus far it has not produced any useful results in biominerals. Tensor tomography gave splendid results on bone,^{99–103} and holds great promise for enamel, and marine biominerals, but these have not been explored yet. Polarization-dependent imaging contrast mapping (PIC mapping) has produced extensively in biomineralization,^{46,104–127} but it is limited to detecting the orientation of the crystalline *c*-axis, it cannot provide the *a*- and *b*-axis orientation, and it is only available at selected synchrotrons already equipped with a photoemission electron microscope (PEEM) and an elliptically polarizing undulator (EPU). Fortunately,

nearly all synchrotrons in every country have a PEEM and an EPU, as these are quite useful for research on magnetic materials. One advantage of PIC mapping is the spatial resolution, which is 20 nm,^{128,129} as opposed to EBSD's 200 nm. Another advantage is that PIC mapping can easily analyze enamel, which is inaccessible to EBSD. The third advantage of PIC mapping, compared to x-ray diffraction methods, is the surface sensitivity: 3 nm at the Ca L-edge (where enamel is measured) and 5 nm at the O K-edge (where calcite, aragonite, and vaterite are measured). Thus, when a 20 nm \times 20 nm \times 3 nm pixel contains the full polarization spectrum, the spectrum is reliably from one crystallite, or two at grain boundaries, not thousands of crystallites as in micro- or nano-diffraction of a bulk biomineral. Thus, PIC mapping results are mathematically tractable.

Mesostructure revealed formation mechanisms

One of the first discoveries based on the mesostructure of biominerals was that amorphous nanoparticles first attach to one another and then crystallize,⁴⁶ as opposed to oriented attachment of particles that crystallize first, and then attach.^{64,130–133} Polycrystalline systems formed by oriented attachment, called mesocrystals, were observed extensively in synthetic materials, but not conclusively in biominerals.^{67,134–138}

In the calcite prisms of mollusk shells, the mesostructure demonstrated that the crystal lattice gradually tilts and suddenly splits within prisms.^{115,139,140}

In nacre, the mesostructure revealed that tablets grow near-epitaxially,^{114,141} that nacre tablet thickness correlates with the temperature at which the shell formed,¹⁴² and that plastic deformation of stacks of co-oriented aragonite tablets cooperatively toughen the material.¹²³ Figure 2 shows the mesostructure of nacre through time.

In enamel, the mesostructure showed that, unexpectedly, in whisker-shaped nanocrystals the elongation direction and the crystalline *c*-axis are not aligned, as they always are in synthetic calcium phosphate whisker crystals. The mesostructure of adjacent whisker crystals, parallel in morphology but forming small-angle boundaries with one another, toughens enamel.¹²¹ Figure 3 shows the mesostructure of human enamel and the small misorientation of adjacent crystals.

In coral skeletons, the mesostructure enabled the discovery that sprinkles, that is, equant, randomly oriented nanocrystals are the first to nucleate in spherulites.¹²⁶ Figure 4 shows the spherulitic mesostructure of coral skeletons.^{119,126} The spherulitic fibers start from sprinkles, which can be either at the growth fronts of spherulites or in the centers of calcification, as observed in different coral species presented in Figure 5. The same mechanism is expected to lead to spherulite formation in other systems besides coral skeletons known to grow spherulitically. These include polymers, organic molecules in food or drugs, or geologic minerals.

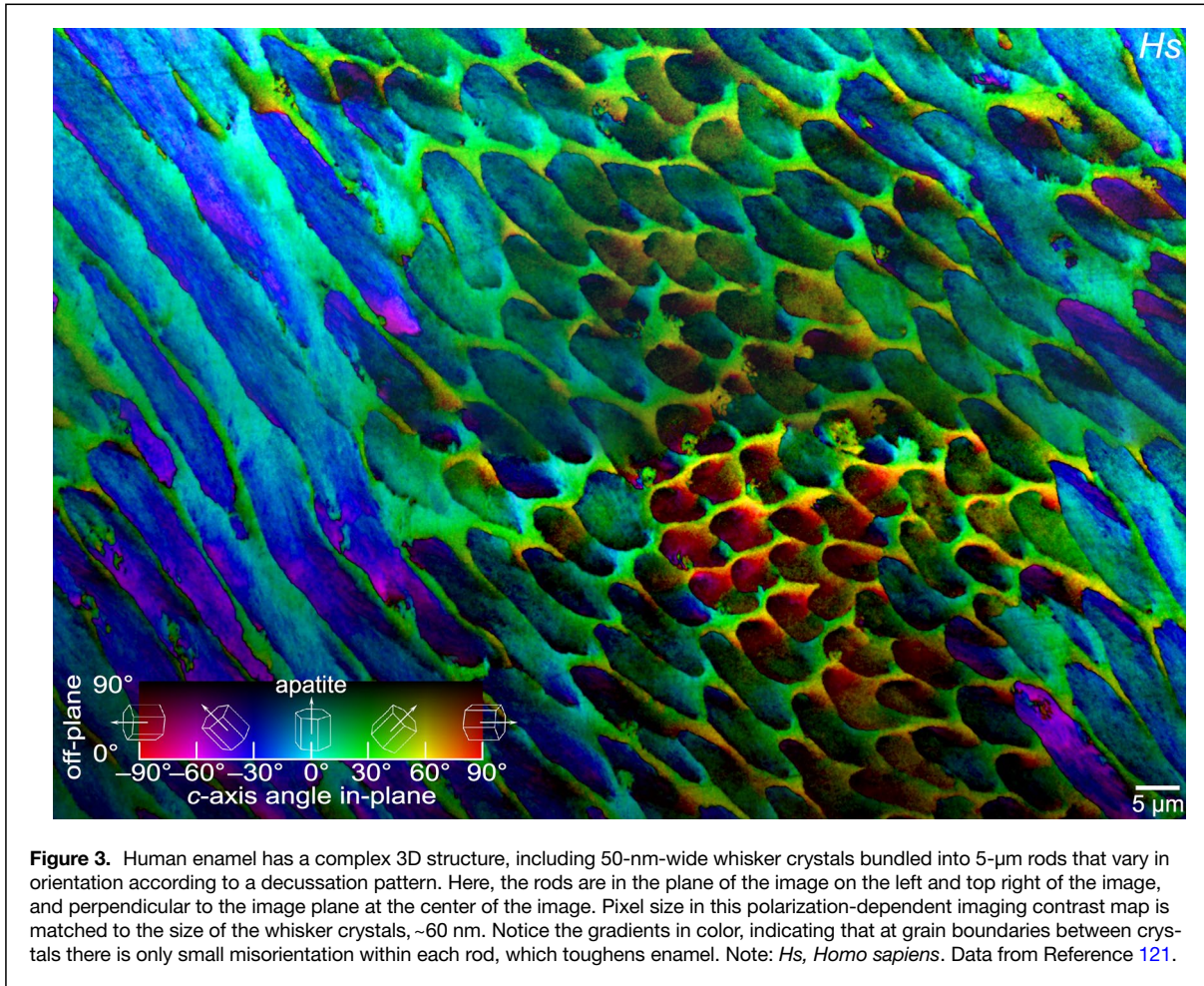


Figure 3. Human enamel has a complex 3D structure, including 50-nm-wide whisker crystals bundled into 5- μm rods that vary in orientation according to a decussation pattern. Here, the rods are in the plane of the image on the left and top right of the image, and perpendicular to the image plane at the center of the image. Pixel size in this polarization-dependent imaging contrast map is matched to the size of the whisker crystals, ~ 60 nm. Notice the gradients in color, indicating that at grain boundaries between crystals there is only small misorientation within each rod, which toughens enamel. Note: *Hs*, *Homo sapiens*. Data from Reference 121.

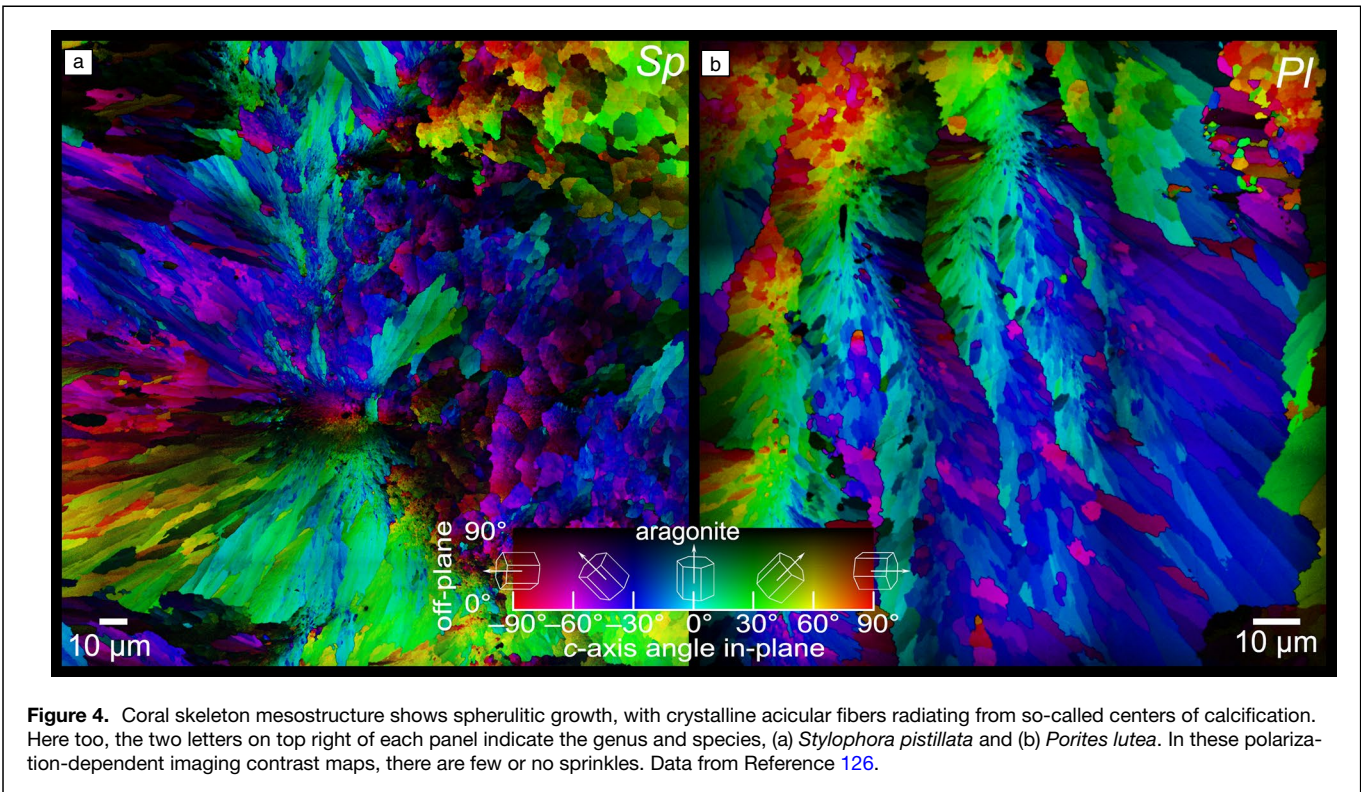


Figure 4. Coral skeleton mesostructure shows spherulitic growth, with crystalline acicular fibers radiating from so-called centers of calcification. Here too, the two letters on top right of each panel indicate the genus and species, (a) *Stylophora pistillata* and (b) *Porites lutea*. In these polarization-dependent imaging contrast maps, there are few or no sprinkles. Data from Reference 126.

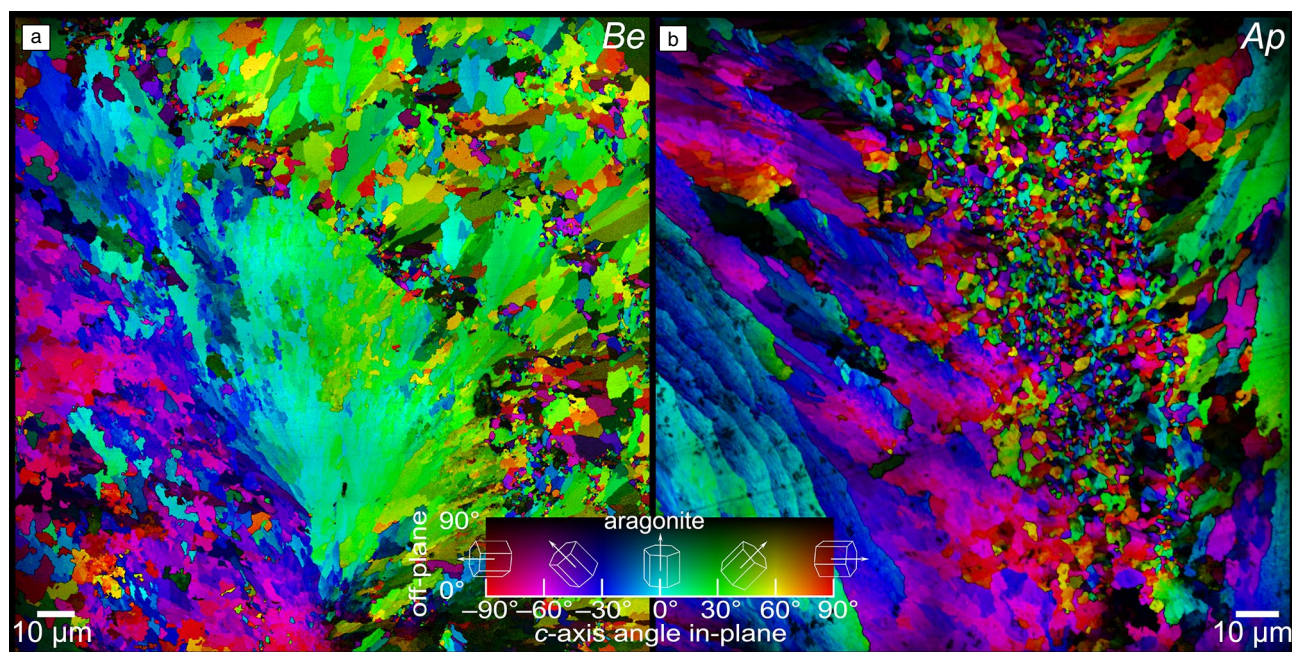


Figure 5. Polarization-dependent imaging contrast maps of coral skeleton from *Balanophyllia europea* (Be) and *Acropora pharaonis* (Ap), two very different coral skeletons that retain sprinkles even after they are mature and fully crystallized. In Be, sprinkles are at growth fronts of spherulites (a), in Ap, they are localized at the centers of calcification (b). Data from Reference 126.

Important questions thus far unanswered

1. What controls the curvature of shells, and shell morphogenesis in general? It is possible that mesostructure at different locations in a shell, corresponding to different developmental stages, will provide the answer.
2. In forming columnar nacre, how do ions or particles reach the growing tablets sites? The pores in the organic sheets are too small^{143–147} for particles⁶⁶ to squeeze through them, and there are tens of organic sheets that particles would have to go through^{148,149} from the depositing cell to the tablet growth site.^{148,149} It must be ions, or a dense liquid precursor⁶² that pass through the pores. But: can ions, easily going through pores of many organic sheets, really diffuse thermodynamically uphill from a less to a more concentrated solution?
3. Why are mollusk shells and sea urchin spines less sensitive than coral skeletons⁶⁵ to anthropogenic-CO₂-induced ocean acidification? The material is precisely the same aragonite or calcite (CaCO₃); thus, their solubility is identical. Does the mesostructure contribute to the solubility of marine biominerals?
4. Can single whisker nanocrystals of human enamel be separated and analyzed by TEM to determine if the crystalline *c*-axis is completely uncorrelated with the whisker elongation direction? PIC mapping suggests so.¹²¹ EBSD of enamel does not work at all, presumably because the whisker crystals are too small, and TEM thus far did not shed light on this key question to understand the single-crystal behavior in tooth enamel. It is simple to obtain

samples from dentists who collect third molars from young adults every day and are usually happy to donate them with minimal paperwork, thus providing an excellent source of healthy human enamel. We use enamel every day when smiling or eating, thus, revealing the crystallography of its fundamental units will provide us with a knowing smile.

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Data availability

All data presented in this review were previously published.

Code availability

The “GG Macros” software used to produce PIC maps can be downloaded free of charge at <https://home.physics.wisc.edu/gilbert/software/>. The GG Macros run on Mac and PC, in Igor Pro 8 (WaveMetrics).

Conflict of interest

The author declares that she has no conflict of interest.

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References

1. H.A. Lowenstam, *Science* **211**, 1126 (1981)
2. H.A. Lowenstam, S. Weiner, *On Biomineralization* (Oxford University Press, Oxford, 1989)
3. A.M. Belcher, X. Wu, R. Christensen, P. Hansma, G. Stucky, D. Morse, *Nature* **381**, 56 (1996)
4. G. Falini, S. Albeck, S. Weiner, L. Addadi, *Science* **271**, 67 (1996)
5. J. Aizenberg, A. Tkachenko, S. Weiner, L. Addadi, G. Hendler, *Nature* **412**, 819 (2001)
6. S. Weiner, L. Addadi, *Science* **298**, 375 (2002)
7. L.A. Touryan, M.J. Lochhead, B.J. Marquardt, V. Vogel, *Nat. Mater.* **3**, 239 (2004)
8. H. Cölfen, *Nat. Mater.* **9**, 960 (2010)
9. J.D. Rimer, Z. An, Z. Zhu, M.H. Lee, D.S. Goldfarb, J.A. Wesson, M.D. Ward, *Science* **330**, 337 (2010)
10. A. Akiva-Tal, S. Kababya, Y.S. Balazs, L. Glazer, A. Berman, A. Sagi, A. Schmidt, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14763 (2011)
11. H. Li, H.L. Xin, M.E. Kunitake, E.C. Keene, D.A. Muller, L.A. Estroff, *Adv. Funct. Mater.* **21**, 2028 (2011)

12. N.A. Sommerdijk, M. Cusack, *Nat. Mater.* **13**, 1078 (2014)
13. S.C. Fitzer, P. Chung, F. Maccherozzi, S.S. Dhesi, N.A. Kamenos, V.R. Phoenix, M. Cusack, *Sci. Rep.* **6**, 21076 (2016)
14. D.E. Jacob, R. Wirth, O.B.A. Agbaje, O. Branson, S.M. Eggins, *Nat. Commun.* **8**, 1265 (2017)
15. A.G. Checa, E. Macías-Sánchez, A.B. Rodríguez-Navarro, A. Sánchez-Navas, N.A. Lagos, *Sci. Rep.* **10**, 16784 (2020)
16. J. Gim, A. Koch, L.M. Otter, B.H. Savitzky, S. Erland, L.A. Estroff, D.E. Jacob, R. Hovden, *Proc. Natl. Acad. Sci. U.S.A.* **118**(42) e2107477118 (2021)
17. S. Mann, C.C. Perry, R.J. Williams, C.A. Fyfe, G.C. Gobbi, G.J. Kennedy, *J. Chem. Soc. Chem. Commun.* (4), 168 (1983). <https://doi.org/10.1039/C39830000168>
18. N. Poulsen, M. Sumper, N. Kröger, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12075 (2003)
19. J. Aizenberg, J.C. Weaver, M.S. Thanawala, V.C. Sundar, D.E. Morse, P. Fratzl, *Science* **309**, 275 (2005)
20. T. Yang, H. Chen, Z. Jia, Z. Deng, L. Chen, E.M. Peterman, J.C. Weaver, L. Li, *Science* **375**(6581), 647 (2022). <https://doi.org/10.1126/science.abj947>
21. L. Li, J.C. Weaver, C. Ortiz, *Nat. Commun.* **6**, 6216 (2015)
22. A.G. Checa, E. Macías-Sánchez, J. Ramírez-Rico, *Sci. Rep.* **6**, 25989 (2016)
23. M.G. Willinger, A.G. Checa, J.T. Bonarski, M. Faryna, K. Berent, *Adv. Funct. Mater.* **26**, 553 (2016)
24. P. Ramos-Silva, D. Wall-Palmer, F. Marlétaz, F. Marin, K.T. Peijnenburg, *J. Struct. Biol.* **213**, 107779 (2021)
25. P.U.P.A. Gilbert, *MRS Bull.* **47**(1), 16 (2022)
26. O. Sibony-Nevo, K. Rechav, V. Farstey, E. Shimoni, N. Varsano, L. Addadi, S. Weiner, *MRS Bull.* **47**(1), 18 (2022)
27. K. Berent, J.H. Cartwright, A.G. Checa, C. Pimentel, P. Ramos-Silva, C.I. Sainz-Diaz, *Phys. Rev. Mater.* **6**, 105601 (2022)
28. A.G. Checa, C. Pimentel, K. Berent, P. Ramos-Silva, A.B. Rodríguez-Navarro, J.H.E. Cartwright, C.I. Sainz-Diaz, *MRS Bull.* **47**, 1 (2022). <https://doi.org/10.1557/s43577-022-00418>
29. C. Orme, A. Noy, A. Wierzbicki, M. McBride, M. Grantham, H. Teng, P. Dove, J. De Yoreo, *Nature* **411**, 775 (2001)
30. J. Aizenberg, *Adv. Mater.* **16**, 1295 (2004)
31. S. Elhadji, J. De Yoreo, J. Hoyer, P. Dove, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19237 (2006)
32. E. Brunner, *Nat. Mater.* **6**, 398 (2007)
33. C. Ortiz, M.C. Boyce, *Science* **319**, 1053 (2008)
34. J. Aizenberg, *MRS Bull.* **35**(4), 323 (2010)
35. D.J. Belton, O. Deschaume, C.C. Perry, *FEBS J.* **279**, 1710 (2012)
36. P.-Y. Chen, J. McKittrick, M.A. Meyers, *Prog. Mater. Sci.* **57**, 1492 (2012)
37. J. Xiao, S. Yang, *Nanoscale* **4**, 54 (2012)
38. U.G. Wegst, H. Bai, E. Saiz, A.P. Tomsia, R.O. Ritchie, *Nat. Mater.* **14**, 23 (2015)
39. H. Du, M. Steinacher, C. Borca, T. Huthwelker, A. Murello, F. Stellacci, E. Amstad, *J. Am. Chem. Soc.* **140**, 14289 (2018)
40. H. Du, E. Amstad, *Angew. Chem. Int. Ed.* **59**, 1798 (2020)
41. H. Zhao, S. Liu, Y. Wei, Y. Yue, M. Gao, Y. Li, X. Zeng, X. Deng, N.A. Kotov, L. Guo, *Science* **375**, 551 (2022)
42. E. Beniash, J. Aizenberg, L. Addadi, S. Weiner, *Proc. R. Soc. Lond. B: Biol. Sci.* **264**, 461 (1997)
43. Y. Politi, T. Arad, E. Klein, S. Weiner, L. Addadi, *Science* **306**, 1161 (2004)
44. Y. Politi, R.A. Metzler, M. Abrecht, B. Gilbert, F.H. Wilt, I. Sagi, L. Addadi, S. Weiner, P.U.P.A. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 17362 (2008)
45. E. Beniash, R.A. Metzler, R.S.K. Lam, P.U.P.A. Gilbert, *J. Struct. Biol.* **166**, 133 (2009)
46. C.E. Killian, R.A. Metzler, Y.T. Gong, I.C. Olson, J. Aizenberg, Y. Politi, F.H. Wilt, A. Scholl, A. Young, A. Doran, M. Kunz, N. Tamura, S.N. Coppersmith, P.U.P.A. Gilbert, *J. Am. Chem. Soc.* **131**, 18404 (2009)
47. M.A. Rivadeneyra, A. Martín-Algarra, M. Sánchez-Román, A. Sánchez-Navas, J.D. Martín-Ramos, *ISME J.* **4**, 922 (2010)
48. U. Wehrmeister, D. Jacob, A. Soldati, N. Loges, T. Häger, W. Hofmeister, *J. Raman Spectrosc.* **42**, 926 (2011)
49. J.H. Cartwright, A.G. Checa, J.D. Gale, D. Gebauer, C.I. Sainz-Diaz, *Angew. Chem. Int. Ed.* **51**, 11960 (2012)
50. Y.U.T. Gong, C.E. Killian, I.C. Olson, N.P. Appathurai, A.L. Amasino, M.C. Martin, L.J. Holt, F.H. Wilt, P.U.P.A. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 6088 (2012)
51. S.E. Wolf, I. Lieberwirth, F. Natalio, J.-F. Bardeau, N. Delorme, F. Emmerling, R. Barrea, M. Kappl, F. Marin, *Faraday Discuss.* **159**, 433 (2012)
52. C.C. Tester, C.H. Wu, M.R. Krejci, L. Mueller, A. Park, B. Lai, S. Chen, C. Sun, M. Balasubramanian, D. Joester, *Adv. Funct. Mater.* **23**, 4185 (2013)
53. A.F. Wallace, L.O. Hedges, A. Fernandez-Martinez, P. Raiteri, J.D. Gale, G.A. Waychunas, S. Whitelam, J.F. Banfield, J.J. De Yoreo, *Science* **341**, 885 (2013)
54. R.T. DeVol, C.-Y. Sun, M.A. Marcus, S.N. Coppersmith, S.C.B. Myneni, P.U.P.A. Gilbert, *J. Am. Chem. Soc.* **137**, 13325 (2015)
55. C. Rodríguez-Navarro, K. Kudlacz, Ö. Cizer, E. Ruiz-Agudo, *CrystEngComm* **17**, 58 (2015)
56. C. Rodríguez-Navarro, E. Ruiz-Agudo, J. Harris, S.E. Wolf, *J. Struct. Biol.* **196**, 260 (2016)

57. A.E. Van Driessche, M. Kellermeier, L.G. Benning, D. Gebauer, *New Perspectives on Mineral Nucleation and Growth: From Solution Precursors to Solid Materials* (Springer, Cham, 2016)
58. S.E. Wolf, C.F. Böhm, J. Harris, B. Demmert, D.E. Jacob, M. Mondeshki, E. Ruiz-Agudo, C. Rodríguez-Navarro, *J. Struct. Biol.* **196**, 244 (2016)
59. E. Macías-Sánchez, M.G. Willinger, C.M. Pina, A.G. Checa, *Sci. Rep.* **7**, 12728 (2017)
60. T. Mass, A.J. Giuffrè, C.-Y. Sun, C.A. Stiffler, M.J. Frazier, M. Neder, N. Tamura, C.V. Stan, M.A. Marcus, P.U.P.A. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **114**, E7670 (2017)
61. C.-Y. Sun, C.A. Stiffler, R.V. Chopdekar, C.A. Schmidt, G. Parida, V. Schoeppler, B.I. Fordyce, J.H. Brau, T. Mass, S. Tambutté, P.U.P.A. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **117**, 30159 (2020)
62. C.A. Stiffler, C.E. Killian, P.U.P.A. Gilbert, *Cryst. Growth Des.* **21**, 6635 (2021)
63. C.A. Schmidt, C.A. Stiffler, E.L. Luffey, B.I. Fordyce, A. Ahmed, G. Barreiro Pujol, C.P. Breit, S.S. Davison, C.N. Klaus, I.J. Koehler, I.M. LeCloux, C. Matute Diaz, C.M. Nguyen, V. Quach, J.S. Sengkhamee, E.J. Walch, M.M. Xiong, E. Tambutté, S. Tambutté, T. Mass, P.U.P.A. Gilbert, *J. Am. Chem. Soc.* **144**, 1332 (2022)
64. J.J. De Yoreo, P.U.P.A. Gilbert, N.A.J.M. Sommerdijk, R.L. Penn, S. Whitelam, D. Joester, H. Zhang, J.D. Rimer, A. Navrotsky, J.F. Banfield, A.F. Wallace, F.M. Michel, F.C. Meldrum, H. Cölfen, P.M. Dove, *Science* **349**, aaa6760 (2015)
65. P.U.P.A. Gilbert, K.D. Bergmann, N. Boekelheide, S. Tambutté, T. Mass, F. Marin, J. Adkins, J. Erez, B. Gilbert, V. Knutson, M. Cantine, J. Ortega Hernandez, A.H. Knoll, *Sci. Adv.* **8**, eabl9653 (2022)
66. P.U.P.A. Gilbert, S.M. Porter, C.-Y. Sun, S. Xiao, B.M. Gibson, N. Shenkar, A.H. Knoll, *Proc. Natl. Acad. Sci. U.S.A.* **116**, 17659 (2019)
67. R.Q. Song, H. Cölfen, *Adv. Mater.* **22**, 1301 (2010)
68. J.D. Currey, *Science* **309**, 253 (2005)
69. H. Imai, "Self-Organized Formation of Hierarchical Structures," in *Biomaterialization I*, ed. by K. Naka, Topics in Current Chemistry Series, vol. 270 (Springer, New York, 2006), p. 43
70. E. Beniash, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **3**, 47 (2011)
71. A.S. Schenk, I. Zlotnikov, B. Pokroy, N. Gierlinger, A. Masic, P. Zaslansky, A.N. Fitch, O. Paris, T.H. Metzger, H. Cölfen, *Adv. Funct. Mater.* **22**, 4668 (2012)
72. X. Liu, K. Lin, C. Wu, Y. Wang, Z. Zou, J. Chang, *Small* **10**, 152 (2014)
73. B. Wang, W. Yang, V.R. Sherman, M.A. Meyers, *Acta Biomater.* **41**, 60 (2016)
74. A.H. Knoll, *Rev. Mineral. Geochem.* **54**, 329 (2003)
75. J.-P. Cuif, Y. Dauphin, J.E. Sorauf, *Biomaterials and Fossils Through Time* (Cambridge University Press, Cambridge, 2010)
76. P.M. Dove, *Elements* **6**, 37 (2010)
77. M. Cusack, *Palaeontology* **59**, 171 (2016)
78. C. McDougall, B.M. Degnan, *Wiley Interdiscip. Rev. Dev. Biol.* **7**, e313 (2018)
79. H. Cölfen, E. Grieshaber, W.W. Schmahl, *Crystals* **11**(3), 299 (2021)
80. T. Douglas, *Science* **299**, 1192 (2003)
81. K. Saruwatari, J. Akai, Y. Fukumori, N. Ozaki, H. Nagasawa, T. Kogure, *J. Mineral. Petrol. Sci.* **103**, 16 (2007)
82. A. Pérez-Huerta, M. Cusack, *Microsc. Microanal.* **15**, 197 (2009)
83. K. Benzerara, N. Menguy, M. Obst, J. Stolarski, M. Mazur, T. Tyliczszak, G.E. Brown Jr., A. Meibom, *Ultramicroscopy* **111**, 1268 (2011)
84. A. Pérez-Huerta, Y. Dauphin, J.P. Cuif, M. Cusack, *Micron* **42**, 246 (2011)
85. A.G. Checa, H. Mutvei, A.J. Osuna-Mascaró, J.T. Bonarski, M. Faryna, K. Berent, C.M. Pina, M. Rousseau, E. Macías-Sánchez, *J. Struct. Biol.* **183**, 368 (2013)
86. X. Yin, E. Grieshaber, A. Checa, F. Nindiyasari-Behal, I. Sánchez-Almazo, A. Ziegler, W.W. Schmahl, *J. Struct. Biol.* **213**, 107707 (2021)
87. C. Sollner, M. Burghammer, E. Busch-Nentwich, J. Berger, H. Schwarz, C. Riekel, T. Nicolson, *Science* **302**, 282 (2003)
88. H.C. Lichtenegger, H. Birkedal, D.M. Casa, J.O. Cross, S.M. Heald, J.H. Waite, G.D. Stucky, *Chem. Mater.* **17**, 2927 (2005)
89. J. Stolarski, R. Przenioslo, M. Mazur, M. Brunelli, *J. Appl. Crystallogr.* **40**, 2 (2007)
90. N. Tamura, P.U.P.A. Gilbert, *Methods Enzymol.* **532**, 501 (2013)
91. A. Gal, K. Kahil, N. Vidavsky, R.T. DeVol, P.U.P.A. Gilbert, P. Fratzl, S. Weiner, L. Addadi, *Adv. Funct. Mater.* **24**, 5420 (2014)
92. X. Chen, C. Dejoie, T. Jiang, C.-S. Ku, N. Tamura, *MRS Bull.* **41**(6), 445 (2016)
93. N.K. Wittig, J. Palle, M. Østergaard, S. Frølich, M.E. Birkbak, K.M. Spiers, J. Garvoet, H. Birkedal, *ACS Nano* **13**, 12949 (2019)
94. J. Palle, N.K. Wittig, A. Kubec, S. Niese, M. Rosenthal, M. Burghammer, T.A. Grünwald, H. Birkedal, *J. Struct. Biol.* **212**, 107631 (2020)
95. V. Chamard, *Acta Crystallogr. Sect. A* **77**, C32 (2021)
96. Y.H. Lo, J. Zhou, A. Rana, D. Morrill, C. Gentry, B. Enders, Y.-S. Yu, C.-Y. Sun, D.A. Shapiro, R.W. Falcone, *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2019068118 (2021)
97. V. Schoeppler, M.A. Marcus, Y.-S. Yu, R.S. Celestre, K.C. Bustillo, R. Falcone, D.A. Shapiro, "Soft X-Ray Linear Dichroic Ptychography: The Study of Crystal Orientation in Biomaterials," in *X-Ray Nanoimaging: Instruments and Methods V*, Proc. SPIE **11839**, ed. by B. Lai, A. Somogyi (SPIE, the International Society for Optics and Photonics, Bellingham, 2021), p. 33
98. J. Walker, G. Langer, *Acta Biomater.* **125**, 83 (2021)
99. M. Liebi, M. Georgiadis, A. Menzel, P. Schneider, J. Kohlbrecher, O. Bunk, M. Guizar-Sicairos, *Nature* **527**, 349 (2015)
100. J. Vogel, F. Schaff, A. Fehringer, C. Jud, M. Wiecezorek, F. Pfeiffer, T. Lasser, *Opt. Express* **23**, 15134 (2015)
101. T.A. Grünwald, M. Liebi, N.K. Wittig, A. Johannes, T. Sikjaer, L. Rejnmark, Z. Gao, M. Rosenthal, M. Guizar-Sicairos, H. Birkedal, *Sci. Adv.* **6**, eaba4171 (2020)
102. M. Liebi, V. Lutz-Bueno, M. Guizar-Sicairos, B.M. Schönbauer, J. Eichler, E. Martinelli, J.F. Löffler, A. Weinberg, H. Lichtenegger, T.A. Grünwald, *Acta Biomater.* **134**, 804 (2021)
103. N.K. Wittig, H. Birkedal, *Acta Crystallogr. B* **78**, 305 (2022)
104. R.A. Metzler, M. Abrecht, R.M. Olabisi, D. Ariosa, C.J. Johnson, B.H. Frazer, S.N. Coppersmith, P.U.P.A. Gilbert, *Phys. Rev. Lett.* **98**, 268102 (2007)
105. P.U.P.A. Gilbert, R.A. Metzler, D. Zhou, A. Scholl, A. Doran, A. Young, M. Kunz, N. Tamura, S.N. Coppersmith, *J. Am. Chem. Soc.* **130**, 17519 (2008)
106. R.A. Metzler, D. Zhou, M. Abrecht, J.-W. Chiou, J. Guo, D. Ariosa, S.N. Coppersmith, P.U.P.A. Gilbert, *Phys. Rev. B* **77**, 064110 (2008)
107. D. Zhou, R.A. Metzler, T. Tyliczszak, J. Guo, M. Abrecht, S.N. Coppersmith, P.U.P.A. Gilbert, *J. Phys. Chem. B* **112**, 13128 (2008)
108. Y.R. Ma, B. Aichmayer, O. Paris, P. Fratzl, A. Meibom, R.A. Metzler, Y. Politi, L. Addadi, P.U.P.A. Gilbert, S. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 6048 (2009)
109. R.A. Metzler, J.S. Evans, C.E. Killian, D. Zhou, T.H. Churchill, N.P. Appathurai, S.N. Coppersmith, P.U.P.A. Gilbert, *J. Am. Chem. Soc.* **132**, 6329 (2010)
110. P.U.P.A. Gilbert, A. Young, S.N. Coppersmith, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11350 (2011)
111. C.E. Killian, R.A. Metzler, Y.U.T. Gong, T.H. Churchill, I.C. Olson, V. Trubetskoy, M.B. Christensen, J.H. Fournelle, F. De Carlo, S. Cohen, J. Mahamid, F.H. Wilt, A. Scholl, A. Young, A. Doran, S.N. Coppersmith, P.U.P.A. Gilbert, *Adv. Funct. Mater.* **21**, 682 (2011)
112. I.C. Olson, P.U.P.A. Gilbert, *Faraday Discuss.* **159**, 421 (2012)
113. I.C. Olson, R. Kozdon, J.W. Valley, P.U.P.A. Gilbert, *J. Am. Chem. Soc.* **134**, 7351 (2012)
114. I.C. Olson, A.Z. Blonsky, N. Tamura, M. Kunz, P.U.P.A. Gilbert, *J. Struct. Biol.* **184**, 454 (2013)
115. I.C. Olson, R.A. Metzler, N. Tamura, M. Kunz, C.E. Killian, P.U.P.A. Gilbert, *J. Struct. Biol.* **183**, 180 (2013)
116. R.T. DeVol, R.A. Metzler, L. Kabalah-Amitai, B. Pokroy, Y. Politi, A. Gal, L. Addadi, S. Weiner, A. Fernandez-Martinez, R. Demichelis, J.D. Gale, J. Ihli, F.C. Meldrum, A.Z. Blonsky, C.E. Killian, C.B. Salling, A.T. Young, M.A. Marcus, A. Scholl, A. Doran, C. Jenkins, H.A. Bechtel, P.U.P.A. Gilbert, *J. Phys. Chem. B* **118**, 8449 (2014)
117. B. Pokroy, L. Kabalah-Amitai, I. Polishchuk, R.T. DeVol, A.Z. Blonsky, C.-Y. Sun, M.A. Marcus, A. Scholl, P.U.P.A. Gilbert, *Chem. Mater.* **27**, 6516 (2015)
118. M.A. Marcus, S. Amini, C.A. Stiffler, C.-Y. Sun, M.J. Frazier, N. Tamura, H.A. Bechtel, D.Y. Parkinson, H.S. Barnard, X.X.X. Zhang, J.Q.I. Chua, A. Miserez, P.U.P.A. Gilbert, *ACS Nano* **11**, 11856 (2017)
119. C.-Y. Sun, M.A. Marcus, M.J. Frazier, A.J. Giuffrè, T. Mass, P.U.P.A. Gilbert, *ACS Nano* **11**, 6612 (2017)
120. C.A. Stiffler, N. Kolln Wittig, M. Sassi, C.-Y. Sun, M.A. Marcus, H. Birkedal, E. Beniash, K.M. Rosso, P.U.P.A. Gilbert, *J. Am. Chem. Soc.* **140**, 11698 (2018)
121. E. Beniash, C.A. Stiffler, C.-Y. Sun, G.S. Jung, Z. Qin, M.J. Buehler, P.U.P.A. Gilbert, *Nat. Commun.* **10**, 4383 (2019)
122. H. Li, C.-Y. Sun, Y. Fang, C.M. Carlson, H. Xu, A. Ješovnik, J. Sosa-Calvo, R. Zarnowski, H.A. Bechtel, J.H. Fournelle, D.R. Andes, T.R. Schultz, P.U.P.A. Gilbert, C.R. Currie, *Nat. Commun.* **11**, 5792 (2020)
123. H.-C. Loh, T. Divoux, B. Gludovatz, P.U.P.A. Gilbert, R.O. Ritchie, F.-J. Ulm, A. Masic, *Commun. Mater.* **1**, 77 (2020)
124. L. Gránásy, L. Rátkai, G.I. Tóth, P.U.P.A. Gilbert, I. Zlotnikov, T. Pusztai, *J. Am. Chem. Soc.* **141**, 1014 (2021)
125. C.A. Stiffler, J. Jakes, J.D. North, D. Green, J.C. Weaver, P.U.P.A. Gilbert, *Acta Biomater.* **120**, 124 (2021)
126. C.-Y. Sun, L. Gránásy, C.A. Stiffler, T. Zaquin, R.V. Chopdekar, N. Tamura, J.C. Weaver, J.A.Y. Zhang, S. Goffredo, G. Falini, M.A. Marcus, T. Pusztai, V. Schoeppler, T. Mass, P.U.P.A. Gilbert, *Acta Biomater.* **120**, 277 (2021)
127. Z. Deng, H.-C. Loh, Z. Jia, C.A. Stiffler, A. Masic, P.U.P.A. Gilbert, R. Shahar, L. Li, *Acta Biomater.* **137**, 147 (2022)
128. G. De Stasio, M. Capozzi, G.F. Lorusso, P.A. Baudat, T.C. Droubay, P. Perfetti, G. Margaritondo, B.P. Tonner, *Rev. Sci. Instrum.* **69**, 2062 (1998)
129. G. De Stasio, L. Perfetti, B. Gilbert, O. Fauchoux, M. Capozzi, P. Perfetti, G. Margaritondo, B.P. Tonner, *Rev. Sci. Instrum.* **70**, 1740 (1999)
130. R.L. Penn, J.F. Banfield, *Am. Mineral.* **83**, 1077 (1998)
131. R.L. Penn, J.F. Banfield, *Science* **281**, 969 (1998)
132. R.L. Penn, J.F. Banfield, *Geochim. Cosmochim. Acta* **63**, 1549 (1999)
133. J.F. Banfield, S.A. Welch, H. Zhang, T.T. Ebert, R.L. Penn, *Science* **289**, 751 (2000)
134. H. Cölfen, M. Antonietti, *Angew. Chem. Int. Ed.* **44**, 5576 (2005)
135. H. Cölfen, M. Antonietti, *Mesocrystals and Nonclassical Crystallization* (Wiley, Hoboken, 2008)
136. Y.-Y. Kim, A.S. Schenk, J. Ihli, A.N. Kulak, N.B. Hetherington, C.C. Tang, W.W. Schmahl, E. Grieshaber, G. Hyett, F.C. Meldrum, *Nat. Commun.* **5**, 4341 (2014)

137. L. Bergstrom, E.V. Sturm, G. Salazar-Alvarez, H. Cölfen, *Acc. Chem. Res.* **48**, 1391 (2015)
138. E.V. Sturm, H. Cölfen, *Crystals* **7**, 207 (2017)
139. A.G. Checa, J.T. Bonarski, M.G. Willinger, M. Faryna, K. Berent, B. Kania, A. González-Segura, C.M. Pina, J. Pospiech, A. Morawiec, *J. R. Soc. Interface* **10**(86), 20130425 (2013)
140. V. Schoeppler, D. Stier, R.J. Best, C. Song, J. Turner, B.H. Savitzky, C. Ophus, M.A. Marcus, S. Zhao, K. Bustillo, *Adv. Mater.* **33**, 2101358 (2021)
141. T.E. Schäffer, C. Ionescu-Zanetti, R. Proksch, M. Fritz, D.A. Walters, N. Almqvist, C.M. Zarella, A.M. Belcher, B.L. Smith, G.D. Stucky, *Chem. Mater.* **9**, 1731 (1997)
142. P.U.P.A. Gilbert, K.D. Bergmann, C.E. Myers, M.A. Marcus, R.T. DeVol, C.-Y. Sun, A.Z. Blonsky, E. Tamre, J. Zhao, E.A. Karan, N. Tamura, S. Lemer, A.J. Giuffre, G. Giribet, J.M. Eiler, A.H. Knoll, *Earth Planet. Sci. Lett.* **460**, 281 (2017)
143. F. Song, A. Soh, Y. Bai, *Biomaterials* **24**, 3623 (2003)
144. M. Rousseau, E. Lopez, P. Stempfflé, M. Brendlé, L. Franke, A. Guette, R. Naslain, X. Bourrat, *Biomaterials* **26**, 6254 (2005)
145. F. Nudelman, B.A. Gottliv, L. Addadi, S. Weiner, *J. Struct. Biol.* **153**, 176 (2006)
146. M.I. Lopez, P.E.M. Martinez, M.A. Meyers, *Acta Biomater.* **10**, 2056 (2014)
147. A. Katsman, I. Polishchuk, B. Pokroy, *Faraday Discuss.* **235**, 433 (2022). <https://doi.org/10.1039/D1FD00111F>
148. H. Nakahara, *Venus (Jpn. J. Malacol.)* **38**(3), 205 (1979)
149. H. Nakahara, "Calcification of Gastropod Nacre," in *Biomaterialization and Biological Metal Accumulation* (Springer, Dordrecht, 1983), p. 225 □

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