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Nanoarchitected Tough Biological Composites from Assembled Chitinous Scaffolds

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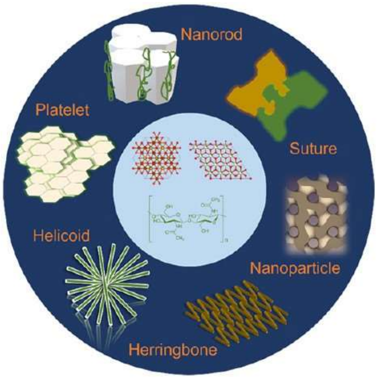
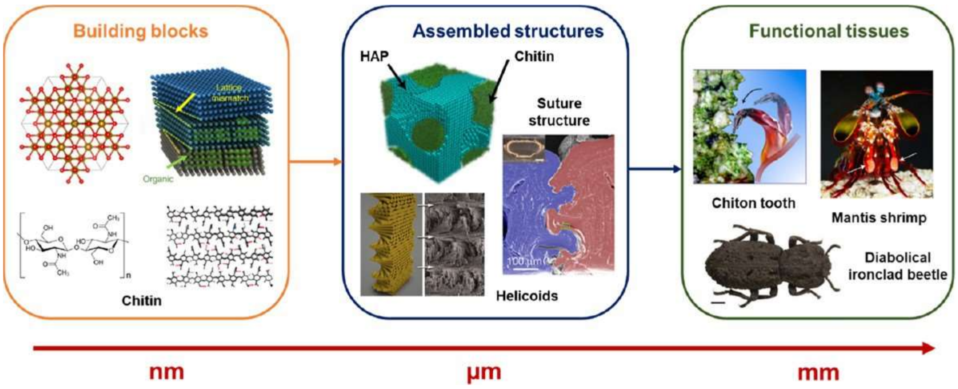


CONSPECTUS: Over hundreds of millions of years, organisms have derived specific sets of traits in response to common selection pressures that serve as guideposts for optimal biological designs. A prime example is the evolution of toughened structures in disparate lineages within plants, invertebrates, and vertebrates. Extremely tough structures can function much like armor, battering rams, or reinforcements that enhance the ability of organisms to win competitions, find mates, acquire food, escape predation, and withstand high winds or turbulent flow. From an engineering perspective, biological solutions are intriguing because they must work in a multifunctional context. An organism rarely can be optimally designed for only one function or one environmental condition. Some of these natural systems have developed well-orchestrated strategies, exemplified in the biological tissues of numerous animal and plant species, to synthesize and construct materials from a limited selection of available starting materials. The resulting structures display multiscale architectures with incredible fidelity and often exhibit properties that are similar, and frequently superior, to mechanical properties exhibited by many engineered materials. These biological systems have accomplished this feat through the demonstrated ability to tune size, morphology, crystallinity, phase, and orientation of minerals under benign processing conditions (i.e., near- neutral pH, room temperature, etc.) by establishing controlled synthesis and hierarchical 3D assembly of nano- to microscaled building blocks. These systems utilize organic−inorganic interactions and carefully controlled microenvironments that enable kinetic control during the synthesis of inorganic structures. This controlled synthesis and assembly requires orchestration of mineral transport and nucleation. The underlying organic framework, often consisting of polysaccharides and polypeptides, in these composites is critical in the spatial and temporal regulation of these processes. In fact, the organic framework is used not only to provide transport networks for mineral precursors to nucleation sites but also to precisely guide the formation and phase development of minerals and significantly improve the mechanical performance of otherwise brittle materials.

Over the past 15 years, we have focused on a few of these extreme performing organisms, (Wang et al., *Adv. Funct. Mater.* 2013, *23*, 2908; Weaver et al., *Science* 2012, *336*, 1275; Huang et al., *Nat. Mater.* 2020, *19*, 1236; Rivera et al., *Nature* 2020, *586*, 543) investigating not only their ultrastructural features and mechanical properties but in some cases, how these assembled structures are mineralized. In specific instances, comparative analyses of multiscale structures have pinpointed which design principles have arisen convergently; when more than one evolutionary path arrives at the same solution, we have a good indication that it is the best solution. This is required for survival under extreme conditions. Indeed, we have found that there are specific architectural features that provide an advantage toward survival by enabling the ability to feed effectively or to survive against predatory attacks. In this Account, we describe 3 specific design features, nanorods, helicoids, and nanoparticles, as well as the interfaces in fiber-reinforced biological composites. We not only highlight their roles in the specific organisms but also describe how controlled syntheses and hierarchical assembly using organic (i.e., often chitinous) scaffolds lead to these integrated macroscale structures. Beyond this, we provide insight into multifunctionality: how nature leverages these existing structures to potentially add an additional dimension toward their utility and describe their translation to biomimetic materials used for engineering applications.

Scheme 1. Multiscale Assembly of Chitin and Inorganic Constituents in Various Living Organisms[a]



Images were reproduced with permission from ref 2, Copyright 2012 AAAS; ref 3, copyright 2020 Springer Nature; ref 4, copyright 2020 Springer Nature; and ref 24, copyright 2011 American Chemical Society. [a]At the nanoscale, the chitin macromolecules can serve as templates in the biomineralization process; at the microscale, the mineralized or unmineralized chitin fibers further assemble into particles, rods or helicoidal and suture patterns; at the macroscale, the micropatterns play important role in the final functions of living tissues such as chiton teeth, mantis shrimp dactyl clubs and the exoskeleton of the diabolical ironclad beetle.

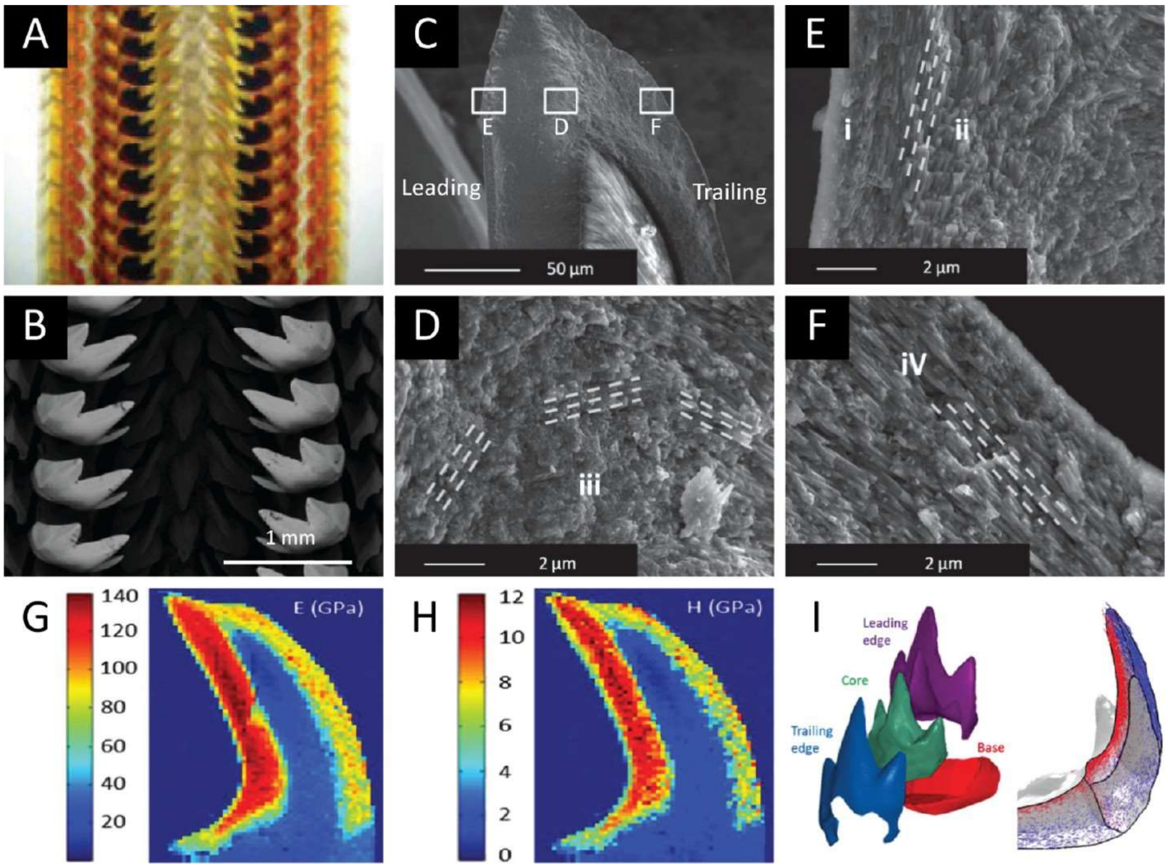


Figure 1. Nanorod structure and mechanical behavior of chiton teeth. (A) Mature section of the chiton radula, displaying major lateral teeth (black). (B) Backscattered SEM micrograph of chiton teeth. (C) SEM micrograph of a longitudinal fracture along a cusp of a chiton tooth showing the rod orientations in different locations (D−F). Reduced modulus (G) and hardness (H) maps obtained by nanoindentation along a longitudinal polished section of a mature tooth. (I) Finite element model of a fully mineralized tooth, with defined components on the left and a stress map of a tooth on the right; direction of tensile (red) and compressive (blue) stresses are reported. Images were reproduced with permission from ref 28, copyright 2014 Wiley, and ref 29, copyright 2010 Elsevier.

radular teeth of *Cryptochiton stelleri*. *Adv. Funct. Mater.* 2013, *23*, 2908−2917.[1] *The first detailed description of dynamic phase and microstructural transformations, guided by chitin scaffolds, that occur during mineralization in the radular teeth of C. stelleri.*

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1. INTRODUCTION

Building lightweight, strong, and tough structural materials in an efficient and cost-effective manner has presented challenges throughout human history. With an extreme population boom over the past century, a significant demand for these materials exists in automotive, aerospace, energy, biomedical, and defense sectors. However, with these advancements, environ- mental impacts were largely ignored.[5] Although centuries have been spent looking for engineering solutions, natural systems have leveraged millions of years, fine-tuning synthetic methods to provide robust yet elegant and precise multiscale structures that have yielded incredible performance.[2−4,6−8] Many of these biological structural materials utilize mineral components to enhance stiffness and hardness yet, unlike their geological counterparts, show incredible toughness that can be attributed to the multiscale features, from the atomic and molecular level to the morphology and material arrangements at the millimeter scale level.[9] These natural designs have afforded significant development of bioinspired designs for strong and tough engineered materials.[10−15]

Thus, understanding how these structures are fabricated and deriving bottom-up synthesis strategies are critical for the next stages of bioinspired materials. Natural systems utilize limited elemental resources to synthesize and assemble organic frameworks with inorganic components under ambient temperature and near-neutral pH conditions to yield multiscale biocomposites.[16−22] One of the most important and abundant organic building blocks in natural materials is chitin, which has been identified in cell walls of fungi, exoskeletons of arthropods, and mineralized tissues such as shells and radula of mollusks.[23−27] Here, we focus on some representative organisms and tissues whose architectures are significantly influenced by chitin fibers, either mineralized or unmineralized (Scheme 1). The assembly of chitin fibrils and their effects on the biomineralization process of iron oxide and calcium phosphate at the nanoscale will be discussed. Various microstructured architectures, formed by chitin fibers and mineral crystals, which play important roles in realizing either mechanical or other related functionalities, will be highlighted.

2. NANORODS

Hard tissues utilized in abrading surfaces or energy absorption frequently consist of elongated mineral nanoarchitectures such as lamellae, platelets, or rods. We have revealed nanorod-based architectures in the teeth of chitons (Figure 1A,B), mollusks that feed on hard rocks to collect food (i.e., algae). Thus, the structure and composition of their teeth have been optimized to provide astonishing abrasion resistance and damage tolerance.

1. Nanorod Architecture in Chiton Teeth

Nanorods have been found in the teeth of many species of chiton, one of which is *Cryptochiton stelleri* or the gumboot chiton. The cusps of its teeth consist of a hard crystalline shell composed of magnetite nanorods and a soft, poorly crystalline, and organic-rich core of iron phosphate. The magnetite nanorods in the shell run parallel to the tooth surface, aligned with the long axis of the tooth and curve around its tip (Figure 1C−F).[28] The diameters of the rods vary from 162 ± 22 nm on the leading edge to 194 ± 30 nm on the trailing edge.[1] Each of these nanorods have high surface roughness, as they are decorated with nanoparticles (i.e., nanoasperities), some of which form mineral bridges that connect adjacent nanorods. Interestingly, similar features have also been observed in the nacreous platelets in the shell of the California red abalone. Both these features are likely a consequence of crystal growth during the biomineralization of the matrix (see section 2.3). Fractured surfaces reveal a conchoidal fracture of the rods with smooth and occasionally bipyramidal faceted surfaces.[28] This is coherent with multifaceted magnetite crystals (about 30 nm) observed in early stages of development of the teeth, which then fuse together to form a single nanorod.

2. Mechanical Behavior of Nanorod Architectures

The magnetite nanorods in *C. stelleri* tooth show a hardness (9−12 GPa) 29 three times higher than human enamel (Figure 1H). The Young's modulus associated with these nanorods decreases by about 15% moving from the leading edge to the trailing edge of the tooth (Figure 1G). This decrease in stiffness is associated with approximately a 20% increase in the rod diameter. Finite element models revealed that, while rasping on a rock, tensile stresses were concentrated on the leading edge of the tooth, while compressive stresses were present on the trailing edge (Figure 1I). Thus, the significantly smaller rod diameters on the leading edge provided higher tensile strength, while the thicker rods lead to compression resistance.

The nanorods, surrounded by an organic matrix of chitinous fibers, lead to cracks propagating preferentially along the rod interfaces, which are critical in creating a tortuous path for cracks to grow and dissipating significant energy. The presence of this organic phase, which has a significantly lower elastic modulus than the mineral, alters stress at the crack tip and thus inhibits crack propagation improving damage tolerance. In *C. stelleri*, cracks were observed to be not only at the interfaces between adjacent rods but also within nanorods, deflected at crystal facet interfaces. In addition, resistance to delamination is enabled by a combination of nanoasperities and mineral bridges that connect adjacent rods.[28] Considering the analogies observed between rods and platelets, rods could be considered as a simplified morphology that the animal uses when unidirectional stresses are involved.

2.3. Biomineralization on Chitin Assembled Structures

The chiton radula is an ideal system to study biomineralization as the whole tooth development process is achieved throughout one entire structure. The initial mineral deposition can be easily identified with optical microscopy (Figure 2A) as

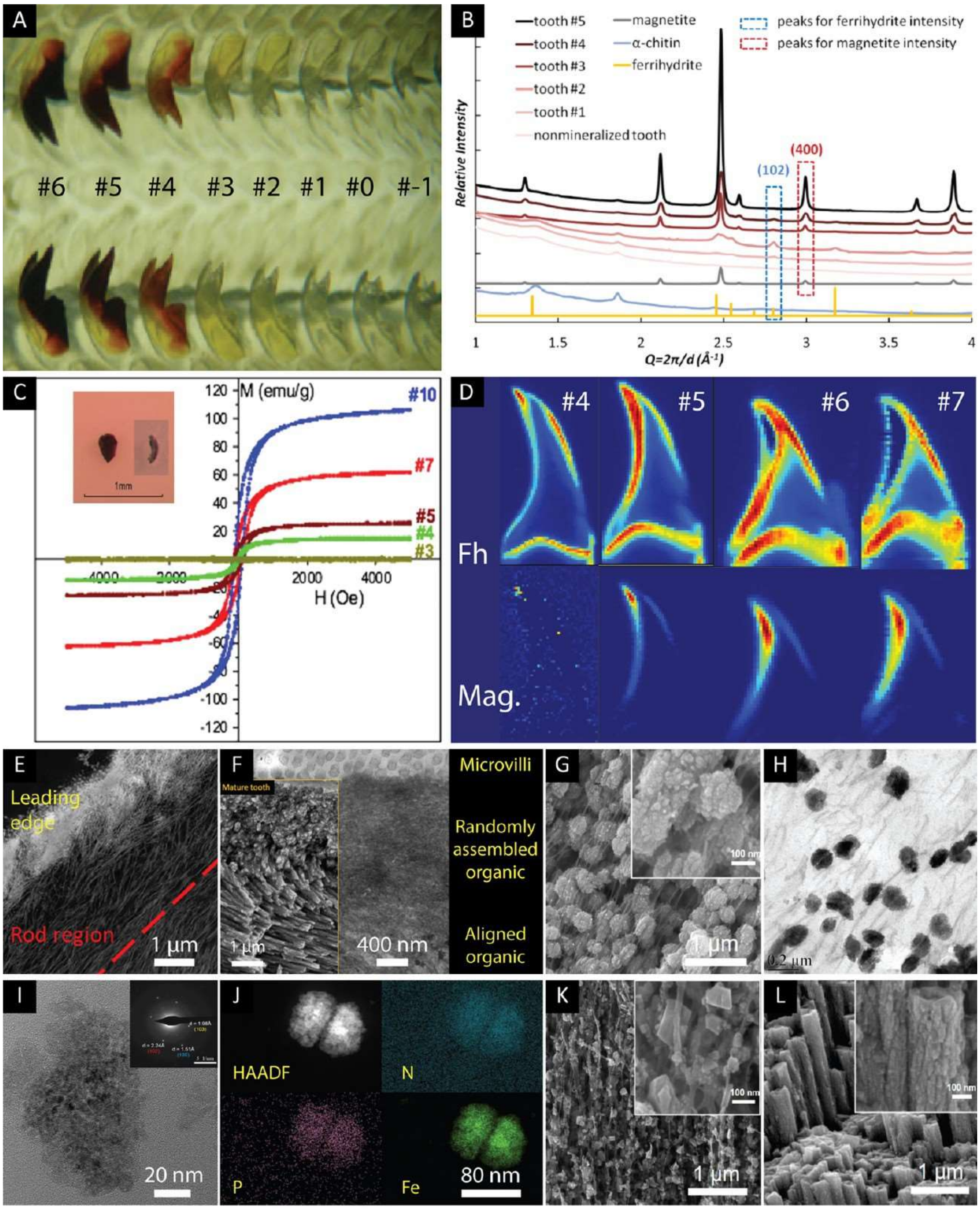

Figure 2. (A) Optical micrograph of immature radula. (B) Synchrotron X-ray diffraction of immature teeth. (C) Magnetometer data demonstrating the onset of magnetization after tooth #3. (D) Phase distribution in immature teeth via μ-XANES analysis. (E) TEM image of tooth #−1 highlighting chitinous scaffold. (F) SEM of fractured leading edge of a mature tooth (left) and TEM image of a similar region in tooth #−1, showing varied chitin scaffold architectures. (G) SEM of mineral particles, guided by chitin nanofibers, within tooth #2 (inset, surface topology).
(H) TEM of mineral particles templated by chitin nanofibers (tooth #2). (I) TEM of a single ferrihydrite nanoparticle (inset, corresponding SAED pattern). (J) Elemental distribution of a particle in tooth #4 via EDS. (K, L) SEM of nanorods from tooth #7 and a mature tooth, with insets
highlighting surfaces where organic fibers surround nanoparticles and nanorods, respectively. Images were reproduced with permission from ref 1, copyright 2013 Wiley, and ref 30, copyright 2022 Wiley.

the color of teeth gradually change from transparent (tooth #−1 and earlier) to yellow and eventually black. Synchrotron X-ray diffraction (Figure 2B) reveals that tooth mineralization starts with the nucleation of ferrihydrite on pre-existing α- chitin fibrils and subsequently converts to magnetite starting at tooth #4 and beyond (Figure 2C,D). The organic matrix (α- chitin with colocated proteins) plays a crucial role in controlling mineral nucleation and growth by modifying the interfacial surface energy for heterogeneous nucleation and crystallization. The assembly of these α-chitin fibrils is different within the tooth (Figure 2E,F), forming a dense, randomly oriented region toward the outermost region of the leading edge of the tooth and a sparse, highly aligned region underneath that guides and compartmentalizes the nanorod growth. The result is a regiospecific architectural design of iron oxide mineral within the mature teeth: a particulate region on the outermost edge (described later in this Account) and nanorod arrays beneath this surface layer. SEM and TEM analyses of a longitudinal section from the leading edge of an immature tooth (tooth #4, Figure 2G,H) show iron oxide particles on highly aligned, chitinous fiber scaffolds. In fact, different dimensions and densities of iron oxide nanoparticles

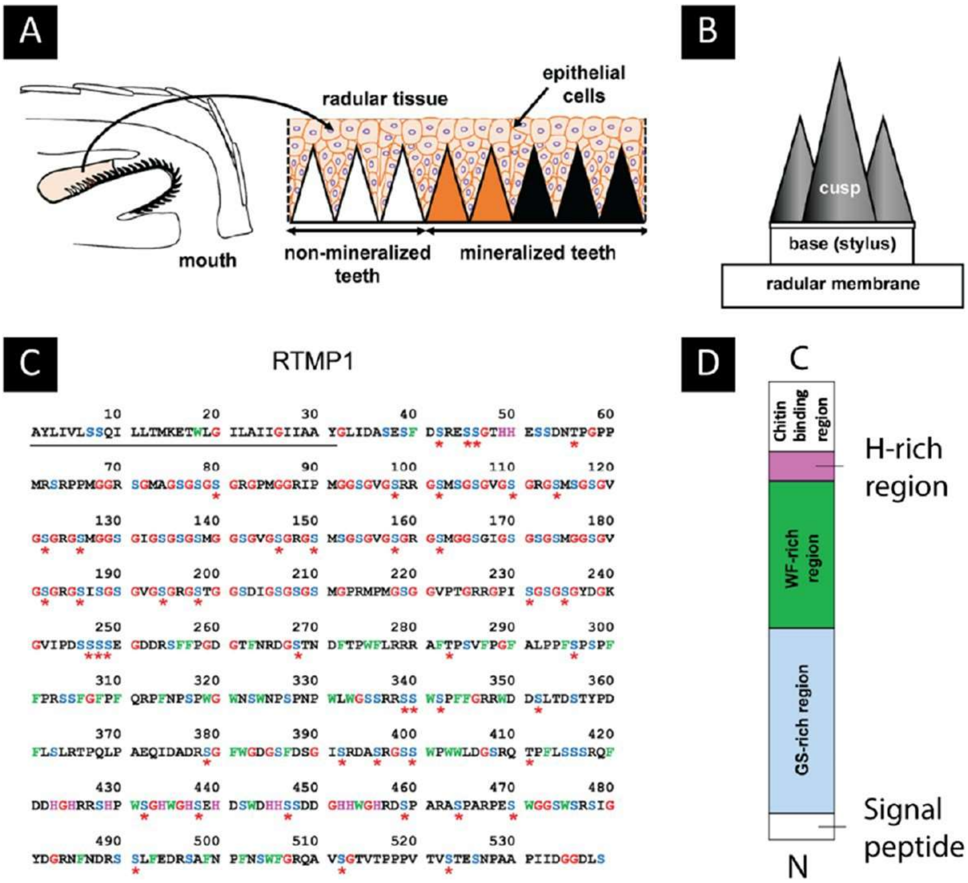

Figure 3. Schematics of radular tissues (A) and an individual radular tooth cusp, chitinous stylus, and base (B) used for proteomic analyses. (C) Amino acid sequence of RTMP1, a cusp-specific radular protein, deduced from cDNA. Asterisks indicate putative phosphorylation sites. (D) Schematic of the domain structure of RTMP1 showing domains with specific repetitive dipeptide. Images were reproduced with permission from ref 32. Copyright 2019 Springer Nature.

have been observed at the leading and trailing edges, ultimately resulting in different final rod diameters.[1] The leading edge, which presents a more densely packed organic matrix (i.e., chitin fibrils) and likely presents more iron-binding sites (i.e., from chitin-associated peptides; see next section), yields a greater number of smaller nanoparticles (ca. 20−50 nm). Conversely, the trailing edge, which contains a less dense chitinous scaffold, develops a lower number of larger mineral aggregates (ca. 100−200 nm), suggesting fewer nucleation sites.

Recent evaluation of these crystal aggregates in early stage teeth revealed ferrihydrite mesocrystals (Figure 2I) with a spherulite-like morphology.[30] We utilized EDS mapping of a mineral particle (Figure 2J) to highlight the presence of not only iron but also nitrogen and phosphorus, which are found in the organic phase. The existence of nitrogen and phosphorus suggests the presence of phosphorylated proteins, which corroborates our previous proteomic analysis of *C. stelleri*[30,31] and suggests that the proteins are colocated with the chitinous fiber scaffolds, which template mineral nucleation. Ultimately, these ferrihydrite particles undergo a solid-state phase transformation to form mesocrystalline magnetite followed by a ripening process to yield well-faceted magnetite crystals (Figure 2K). These separated magnetite particles, which are aligned on the same chitin fiber, eventually fuse to form a continuous nanorod in the fully mature teeth (Figure 2L).

Proteomic analyses of the mineralized tooth cusps (Figures 3A,B) showed the presence of diverse proteins, among which are myoglobin and highly acidic peptides, likely involved in oxygen and iron binding.[31] Further analyses lead to the hypothesis that an acidic Mms6-like protein and RTMP1 (a phosphorylated serine-rich protein, Figures 3C,D), containing different chitin-binding domains, are bonded to the chitin scaffold providing Fe-binding sites to locally increase the iron concentration and act as nucleation points.[32] Despite the strong templating effect from the organic scaffold, different analyses of the tissue surrounding the immature teeth highlighted strong involvement of cells in controlling the mineralization conditions. Among them, cells seem to be responsible for activating the conversion of ferrihydrite to magnetite by transporting Fe^{2+}-based species into the scaffold (a chemical conversion already replicated *in vitro*). This process seems to involve different proteins responsible for iron reduction, such as peroxiredoxin-6-like proteins.[32]

3. HELICOIDS

Besides abrasive resistance, chitin assembled structures with exceptional impact and crush resistance have been observed in living organisms. Helicoidal structures, also known as Bouligand structures, have been found in many animal tissues including shells and appendages of crustaceans and fish scales.[33] Microscale biopolymer fibers (chitin, collagen, cellulose, etc.) align and form lamellae, which are further stacked together with continuous rotation at a certain angle with respect to adjacent layers resulting in multiple 180° periods, which will ultimately form the final bulk tissue.[2,34] One of the main advantages of this structural design in natural materials is its ability to resist impact and crack propagation

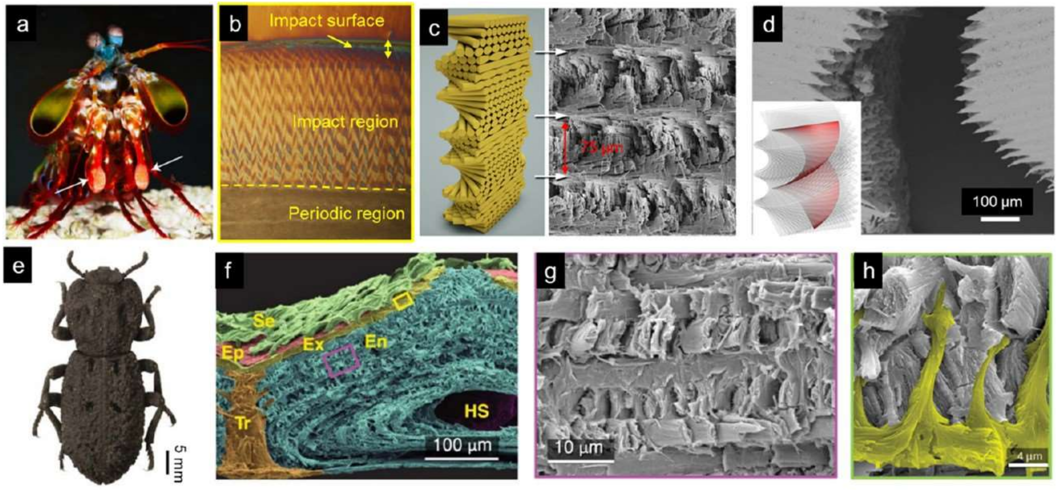

Figure 4. Helicoidal architectures in the mantis shrimp dactyl club and cuticle of the diabolical ironclad beetle. (a) Mantis shrimp and its dactyl clubs (white arrows). (b) Optical micrograph of a cross-section of the dactyl club displaying multiple regions. (c) SEM and schematic of helicoidally arranged chitin fibers in the periodic region in panel b. (d) Schematic and SEM image showing the crack twisting by the helicoidal chitin fibers in the dactyl clubs. (e) Image of diabolical ironclad beetle (*P. diabolicus*). (f) False colored SEM micrograph a cross section of beetle elytra. (g) Helicoidal microstructure of chitin fibers in the endocuticle. (h) Interply penetrating chitin microfibers (false colored yellow) within the helicoidal structure. Images were reproduced with permission from ref 2, copyright 2012 AAAS; ref 3, copyright 2020 Springer Nature; ref 4, copyright 2020 Springer Nature; ref 37, copyright 2016 Wiley; ref 38, copyright 2018 Elsevier; and ref 44, copyright 2021 Wiley.

leading to catastrophic failures, thus protecting the underneath soft tissues efficiently and increasing the lifetime of the biological armors or weapons.[2,35,36]

1. Helicoid in Mantis Shrimp

The dactyl club of the mantis shrimp, which can be accelerated to nearly 23 m/s at an acceleration over 10000*g* to impact the shells of various marine prey, is a primary example of a biological composite with a helicoidal architecture that consists of chitinous fibers surrounded by a mineral matrix (Figure 4a). The club can be used in thousands of impacts in a lifecycle yet does not fail. Three regions have been identified in the dactyl club that can help to resist impact and absorb large amounts of energy (Figure 4b).[2] The outer surface layer is the impact surface, which is a layer of highly mineralized hydroxyapatite (HAP) and chitin nanoparticles; the middle region is the impact region, containing mineralized chitin fibers arranged in a helicoidal architecture that displays a herringbone pattern; the inner region is the periodic region, which consists of helicoidally arranged chitin fibers surrounded by an amorphous calcium phosphate matrix (Figure 4c).[2,37] An oscillation of elastic modulus between 10 and 25 GPa in the periodic region has been observed, which is caused by the anisotropy of the chitin fibers. Crack deflection at interfaces created by chitin fibers, as well as twisting that follows the helicoidal structure, was shown in both experiments and theoretical analyses (Figure 4d).[38] Compared to the helicoidal structures, the herringbone arrangement of chitin fibers in the impact region provides additional out of plane stiffness and strength. Higher compressive strength and strain were also observed in herringbone structures.[37]

2. Helicoids in Insects: Beetles

Beyond crustaceans such as the mantis shrimp, multiple examples of organisms adopting fiber-reinforced composite structures with the helicoidal motif are common.[39] Insect cuticles have been widely studied because of their unique and elegant architectures that fulfill the organism's numerous needs for survival.[40,41] In Coleoptera, a transformation of their delicate forewings into protective stiff wings (elytra), which occurred about 350 million years ago,[42] facilitated an evolutionary diversification that resulted in an excess of 350 000 distinct species including flying, terrestrial, and aquatic variants.[43] The elytra ultimately serve as a protective cover for the underlying delicate hind wings.

We recently investigated two species of beetles from different habitats and described how variations in their exoskeletal architecture and composition allow them to thrive in their respective environments.[44] We focused on the elytra from two species of beetles that branched approximately 250 million years ago:[40,42] *Trypoxylus dichotomus* (known as the rhinoceros beetle), a tree-dwelling and thus flight capable beetle inhabiting East Asia, and *Phloeodes diabolicus* (named the diabolical ironclad beetle), a terrestrial fungivore incapable of flight residing on the western coast of North America.[4,45] For *P. diabolicus*, this inability to actuate its elytra has resulted in a structurally robust cuticle capable of withstanding predator strikes (Figure 4e). Our investigation of the microstructures within the innermost region of the elytra, the endocuticle, of both organisms revealed a hierarchical arrangement, with lamellae that contain balkens, or parallel bundles of fibers, each of which is constructed from unidirectional microfibrils.[46] The fibers in one lamella are oriented and rotated in-plane from the adjacent lamella successively, providing isotropy to the elytra.[38] Examination of a fractured surface of the endocuticle from *P. diabolicus* highlights that the fiber orientation between adjacent lamellae changes by 30°, a helicoidal-like architecture (Figure 4f,g) that, once again, provides interfaces (i.e., between chitin fibers and cuticle proteins) for crack deflection as well as twisting. Conversely, the endocuticle from *T. dichotomus* has a rotation of 90° per layer. The incorporation of a greater angular variation between lamellae in the elytra of *T. dichotomus* is likely adopted to maximize isotropy with a minimal number of lamellae to reduce weight and enable flight. Interestingly, a closer observation of the endocuticle in *P. diabolicus* reveals a variation in the helicoid: interply penetrating microfiber strands (i.e., *z*-pinning fibers) that connect adjacent lamellae (Figure 4h). These *z*-pinning fibers add vertical support through successive layers and provide

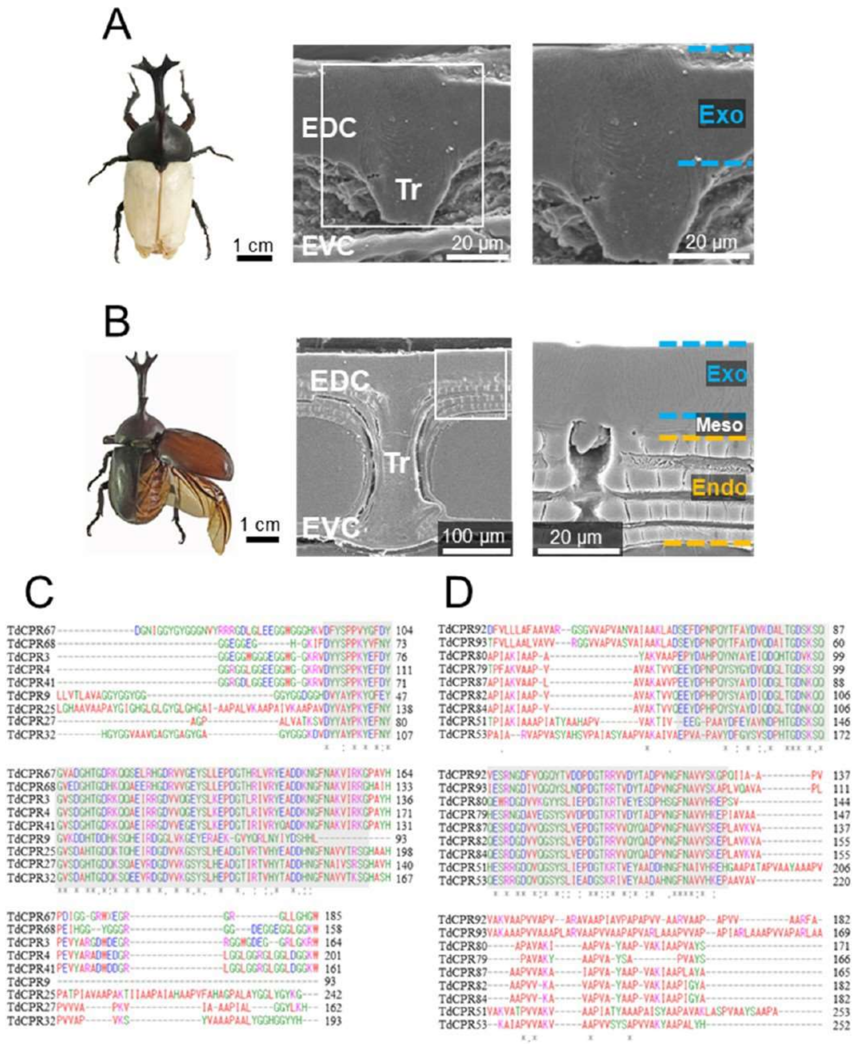

Figure 5. Elytral microstructure of *T. dichotomus* at (A) 0 h after eclosion and (B) 192 h after eclosion. EDC, elytral dorsal cuticle; EVC, elytral ventral cuticle; Tr, trabeculae; Exo, exocuticle; Meso, mesocuticle; Endo, endocuticle. (C) Gly-rich region and (D) multiple Ala-Ala-Pro repeats found in CPs identified from *T. dichotomus*. The region highlighted in gray indicates chitin-binding domains. Images were reproduced with permission from ref 49. Copyright 2022 Elsevier.

stiffness and toughness to the nonmineralized structure with augmented interlaminar resistance to shear. This is similar to those observed in tooth enamel and ram horns; through thickness these fibers infer common microstructural design themes used for compression resistance.[47,48]

3.3. Self-Assembly of Elytra

To form these robust and elegant structures, beetles undergo a complete metamorphosis, where the larva transforms into a pupa and then to an adult. We have investigated this process in *T. dichotomus*.[49] Immediately after eclosion, the elytra of *T. dichotomus* are pale and soft. Over a period of several days, the elytra become progressively darker and less flexible. The elytron of *T. dichotomus* is composed of dual chitinous layers, a thick dorsal and a thin ventral layer. As the elytron matures, a substantial space develops between these two layers with formation of a number of supporting structures known as trabeculae. The dorsal cuticle of untanned white elytron (0 h after eclosion, 0 HAE) is composed of the exocuticle (Figure 5A). At 96 HAE, the mesocuticle is evident underneath the exocuticle as is the endocuticle, which is composed of thick chitinous laminae that have begun to assemble underneath the mesocuticle eventually forming multiple 0°−90° oriented laminae, which are observed in the elytra at 192 HAE (Figure 5B).

Although *T. dichotomus* does not present a helicoidal architecture, it provides a pathway through which assembly may be understood. A recent molecular study has demon- strated the functional importance of the structural cuticular proteins (CPs) surrounding the chitin fibers. During the structural development of *T. dichotomus* elytra, protein profiles of elytra drastically change. Proteomic study on *T. dichotomus* has revealed characteristic CPs for the structural development of elytra, which are CPs with Gly-rich regions (Figure 5C) and multiple Ala-Ala-Pro (AAP) repeats (Figure 5D).[49] These CPs have chitin-binding sites called Rebers and Riddiford motif. Expression behavior of these CPs were different: CPs containing Gly-rich region were mainly identified only from untanned white elytra. On the other hand, CPs containing AAP repeats were identified from both untanned and tanned elytra. These 2 types of CPs have chitin-binding motifs and may play an important role in chitinous layer formation via controlling of self-assembly of chitin nanofibers. Further functional analysis on these CPs may contribute to understanding these mechanisms.

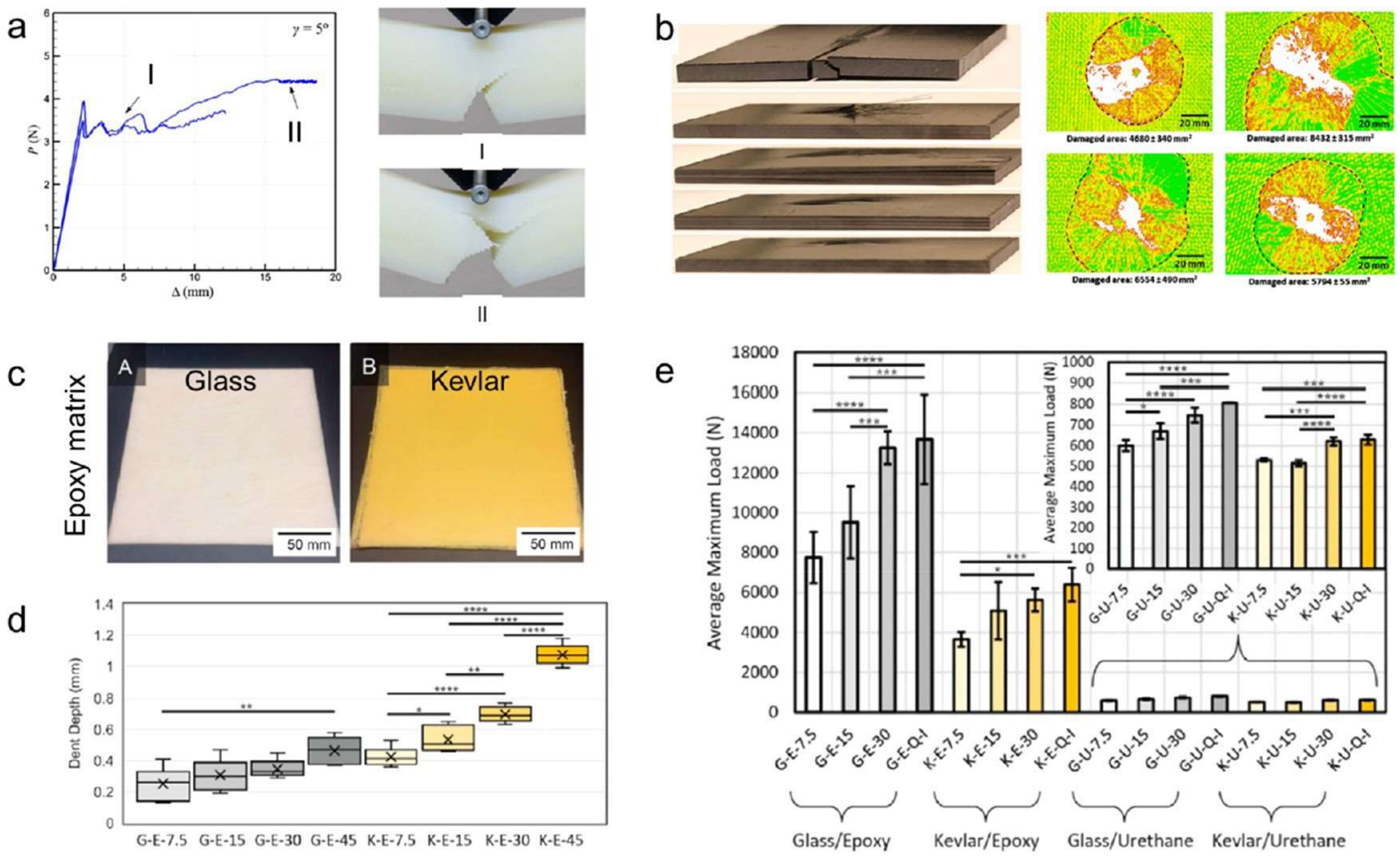

Figure 6. Bioinspired designs of helicoidal composite structures. (a) Experimental result and theoretical analyses of the crack twisting mechanism in helicoidal structure. Three-point bending experiments of 3D printed samples show crack twisting. (b) Drop weight impact tests of carbon fiber reinforced composites (CFRP) with helicoidal structures. Ultrasonic C-scanning images show the internal damages in different CFRP samples. (c) Bioinspired helicoidal glass and Kevlar fiber reinforced epoxy composites. (d) Dent depth in different samples after drop weight impact tests. (e) Average maximum load of different samples in the drop weight tests. Images were reproduced with permission from ref 12, copyright 2014 Elsevier; ref 38, copyright 2018 Elsevier; and ref 50, copyright 2020 Elsevier.

3.4. Biomimetic Implementation of Helicoids

Bioinspired composites based on the helicoidal blueprints provided not only from dactyl clubs of mantis shrimp but also from the elytra of the diabolical iron clad beetle were fabricated and studied.[12,50] 3D printed plastic composites show significant increases in damage tolerance in helicoidal structures compared to unidirectional and quasi-isotropic designs, primarily due to the crack twisting mechanisms (Figure 6a).[38] The impact resistance and damage tolerance of helicoidal structures were also investigated in carbon fiber reinforced plastic (CFRP) composites (Figure 6b).[12] In drop weight tests, helicoidal samples had smaller indentation depths compared with quasi-isotropic controls. The internal damage was more distributed over larger areas in helicoidal samples (Figure 6b). In addition, helicoidal structures made from glass and Kevlar fiber (Figure 6c) were fabricated with different interply rotation angles (7.5°, 15°, 30°, 45°).[50] Drop weight impact tests shows significant decreases in dent depth in the helicoidal samples with lower rotation angles (Figure 6d), while there was more extensive cracking in the quasi-isotropic composites. The quasi-isotropic samples have the highest average peak impact loads compared to the helicoidal designs (Figure 6e). This indicates that the quasi-isotropic designs have higher rigidity and thus would serve in load bearing applications. However, from the damage resistance and energy dissipation point of view, the helicoidal designs show improved performance.

4. NANOPARTICLES

By improving the abrasive and impact resistance behavior of the structures discussed above, coatings constituting nano- particles assembled from chitin macromolecules have been developed by nature to provide further protection, while in various engineering applications thin film coatings consisting of high concentrations of nanoparticles have also been applied as protection for underlying structures from abrasion, impact, and erosion damage.[51,52] One of the representative applications is the coating layers on wind turbine blades, which can be damaged by erosion from rain droplets, sand, and flying particles. Nanoparticle coatings on the blades derived from sol−gel methods can reduce the erosion to a very large extent while having little effect on the weight.[51]

1. Impact Resistant Nanoparticle Coating in Mantis Shrimp

In the previous section, it was demonstrated that helicoidally assembled chitin fibers provide significant damage tolerance to the dactyl clubs of mantis shrimp. Our recent study indicates that an ultrathin coating (∼70 μm) of composite hydrox- yapatite/chitin nanoparticles exists on the outermost surface of the dactyl club.[3] Damage localization and energy dissipation under both low strain rate indentation and high strain rate impacts were observed in this nanoparticle layer. Figure 7a shows a schematic of the nanocomposite coating on the surface of dactyl clubs. The ∼60 nm size hydroxyapatite/chitin composite nanoparticles were determined to have formed by

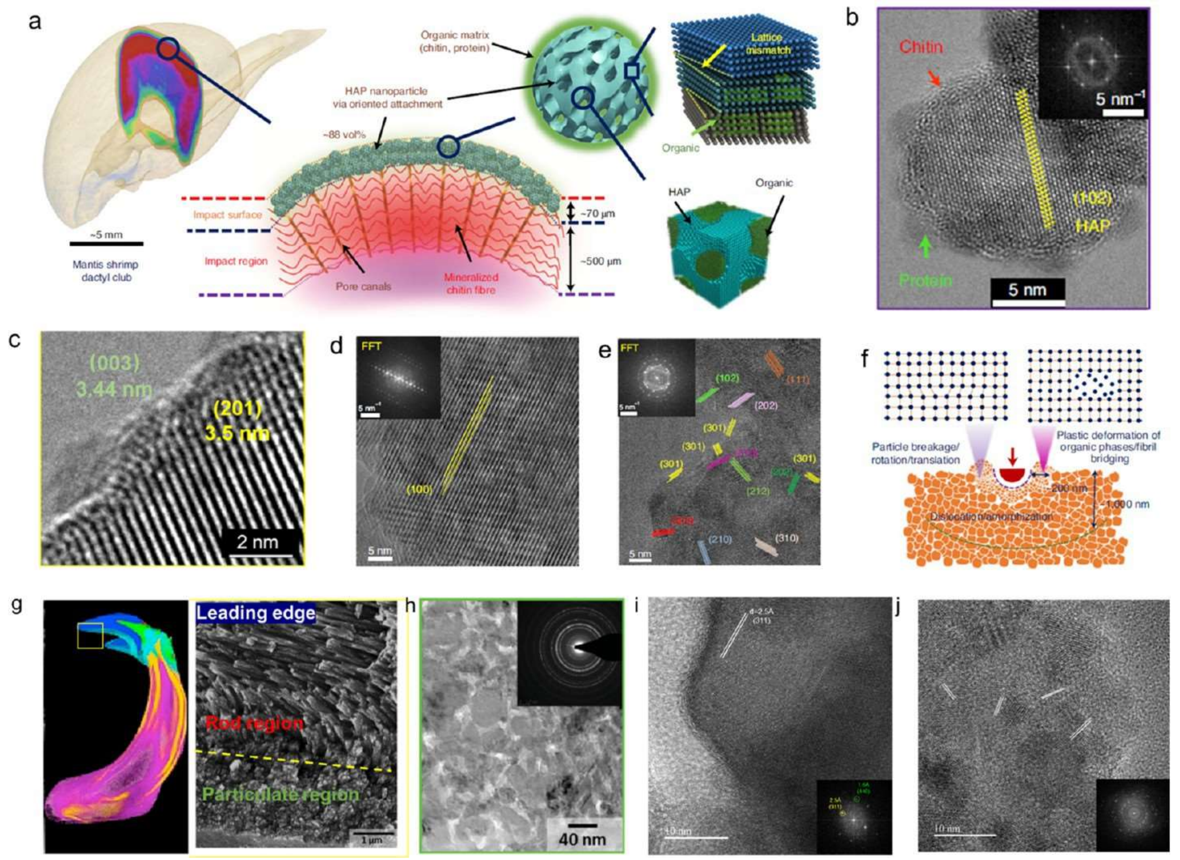

Figure 7. Hydroxyapatite/chitin nanoparticle coating on the surface of the mantis shrimp dactyl club. (a) Schematic of the hierarchical structure of the dactyl club and nanoparticle coating layer. (b) High resolution TEM image of the composite nanoparticle. (c) High resolution TEM image of the interface between the chitin molecule and (201) plane of HAP. (d, e) TEM images of nanoparticle before and after high strain rate impact, respectively. (f) Schematic of the multiscale toughening mechanisms in the coating layer. Images were reproduced with permission from ref 3. Copyright 2020 Springer Nature. (g) Surface analysis of the leading edge of the fully mineralized chiton tooth. SEM of longitudinal fracture surface (yellow box in the tooth schematic), highlighting a 2 μm nanoparticle layer. Schematic image was reproduced with permission from ref 6. Copyright 2020 Elsevier. (h) TEM of FIB-sectioned mature tooth showing details of particulate outer layer, with selected area diffraction confirming the magnetite phase. (i) TEM analysis on a single particle from outer surface layer (inset, FFT suggesting single crystal or mesocrystallinity). (j) Surface nanoparticle transition after scratch (inset, FFT demonstrating polycrystalline particles).

oriented attachment of smaller nanocrystalline hydroxyapatite domains. Organic chitin and protein molecules, which were observed in these particles, serve as templates for the crystallization of hydroxyapatite nanocrystals. Figure 7b shows a high-resolution TEM image of the chitin and proteins attached to HAP (102) planes. Organics were also found occluded in the HAP crystal lattice, modifying the lattice parameters of the HAP crystals (Figure 7c). The interface between a chitinous molecule and the (201) plane of HAP is shown in Figure 7d. The similarity between their *d*-spacings suggested the possibility of an epitaxial relationship. HAP/ chitin nanoparticles, before and after high strain rate impacts, are shown in Figure 7e,f. These high strain rate impacts lead to fracture of the composite nanoparticles providing significant energy absorption to the dactyl club. Other mechanisms such as particle rotation and translation, plastic deformation of organics and dislocation and amorphization were also found in this system (Figure 7f).[3] Bioinspired nanoparticle coatings based on the structural designs found in the mantis shrimp dactyl club surface can be applied to protect brittle and fragile substrates such as crystalline semiconductors and carbon fiber reinforced composites. This will be covered in our future work.

4.2. Wear Resistant Nanoparticle Coating in Chiton Teeth

Similar to the mantis shrimp's dactyl club, we observed a nanoparticle coating on the surface of mature chiton (*C. stelleri*) teeth. Here, it is likely that these protective coatings could be an evolutionary common design theme. SEM of the fractured mature tooth from *C. stelleri* (Figure 7g) revealed a 2 μm dense layer of nanoparticles covering the underlying nanorod architectures. TEM analysis on this particulate region (Figure 7h) shows a wide particle size distribution (8−60 nm) of magnetite nanoparticles with random orientations relative to each other. Additional analyses (Figure 7i,j) of abraded particles revealed both intact single crystal (or mesocrystalline) particles and what appear to be fractured polycrystalline magnetite particles, which may have been induced by the abrasive forces. Thus, we consider this may provide a similar energy absorption mechanism, specifically for enabling wear- resistance during feeding on hard surfaces.

5. DISCUSSION AND PERSPECTIVES

In this Account, we have presented a few key design features, nanorods, helicoids, and nanoparticles, observed in different organisms that evolved to survive under extreme conditions. In these structures, underlying chitinous scaffolds are often coassembled with peptides as well as inorganic species via precisely controlled mineralization processes, which lead to multiscale biological composite materials that demonstrate incredible performance. Although we describe these architec- tures in specific organisms investigated in our lab, these blueprints are found in many other species, suggesting a convergence of an optimal biological design. It also enables us to leverage these blueprints to develop biomimetic structures that utilize engineered materials to translate to high perform- ance structures.

Although we, and others, have made significant progress in this area, several key challenges remain.

(i) Beyond the architectural features we described above, the interfaces afforded by these chitinous scaffolds continue to be an exciting area for advancement of materials science that can be used to help in joining dissimilar materials, such as plastics and metals, in engineered structures, which currently remains a challenge. In our recent work investigating the diabolical iron clad beetle,[10] we revealed that the two halves of this protective armor were joined by a medial suture. The interdigitated, puzzle piece-like features of this suture consist of chitinous fibers wrapped circumferentially around bulbous lobes that provide strength and toughness to avoid catastrophic failure. Biomimetic composites were fabricated that d emonstrated an immediate benefit over aviation fasteners, providing enhanced strength and significantly increased toughness.

(ii) Clearly, there is a need to have a deeper understanding of how the chitinous scaffolds, which guide these key architectures, assemble, with functional proteins to form the framework that determines their resultant structures. We have investigated, for example, the assembly of the elytra from one species of beetle and are now beginning to look at others. One of the challenges here is collecting specimens from organisms whose growth cycle is difficult to control in the laboratory. One path that can be used to circumvent this issue is *in vitro* fabrication of structures using peptides extracted from organisms to guide the assembly of suspensions of chitinous fibers. However, there are clearly more variables that dictate this assembly process and more work is needed here. Utilization of colloidal-based liquid crystals where chemically modified surfaces of anisotropic particles orfibers like chitin, under controlled conditions such as tuned surface chemistry, pH, ionic strength, as well asrate of water removal during assembly, can be leveraged to form large scaled self-assembled periodic structures[53,54] and may enable further discovery of biological mechanisms (i.e., validation) as well as translation to engineered structures. Beyond the assembly of the scaffolds, understanding the control mechanisms of mineralization, including the type of inorganic species that are transported (and how they are transported) to these assembled nucleation sites is still poorly under- stood. Clearly, revealing some of these mechanisms will also enable finer control of bioinspired composite structures as well the potential for development of controlled green synthesis of more challenging materials.

(iii) Many of these biological structures are multifunctional, serving more than just a structural purpose. For example, in the mantis shrimp, we observe chitinous and vertically aligned pore canals that not only act to deter delamination of helicoidal layers in the dactyl club but also provide out of plane stiffness to enable momentum transfer to its prey. Beyond this, these pore canals are suggestive of mineralization pathways in the organism that could be utilized in self-healing composite materials. These tubular structures have been observed in the cuticles of beetles, where a similar *z*-pinning effect would benefit survival. Of course, there are more functionalities to be uncovered and explored in many organisms.

(iv) Many of these organisms have deviations in their structures and functions that are dictated by their environment and ecological constraints. Variations in these organisms, as well as the environments within which they were derived will be further investigated. This will be important to extrapolating new, non-native designs that may provide utility in more extreme environments.

(v) Finally, we look to leverage additive manufacturing techniques to build scalable structures that can imple- ment non-native designs and multifunctionality. How- ever, integrating some of the assembly mechanisms used by natural systems, as well as those described in point ii above, into manufacturing platforms remains a large but not insurmountable challenge.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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