BIOMATERIALS FOR SMALL TOOLS

The jaws, leg claws, stings and other "tools" of a large fraction of arthropods, some worms and members of other phyla, contain extraordinary amounts of heavy elements (e.g. Zn, Mn, Cu, and Br). Although the concentrations reach 25% of dry mass, the material is not filled with a biomineral, like calcified tissues are. Instead, Heavy Element Biomaterials (HEBs) appear to be a distinctly different system that is more widely employed among small organisms and which may inform humans as we build microscopic tools.

Here we propose to test hypotheses related to the mechanical properties and composition of the HEBs: 1) HEBs are particularly fracture resistant and, 2) the different ones provide different balances of hardness and fracture resistance, 3) consistent with the tool regions in which they are employed, 4) that they do this by controlling bound water, 5) that they are particularly resistant to impact fracture because of the inertial properties of the heavy elements, and 6) that the Zn in Zn HEBs is bound to histidine and hydroxide.

In our previous NSF-funded project (IOS 0422234), we compared the mechanical properties of a Br-rich HEB that we found at the tips of certain crab claws, to the calcified cuticle in the rest of the claw (Schofield *et al*., 2009). We measured modulus of elasticity, hardness, energy of fracture, abrasion resistance, and loss and storage moduli. Most of these properties had not previously been measured for any HEB. We modified nano-indentation techniques for some of these measurements and built instruments to measure energy of fracture and abrasion resistance for small specimens. The greatest advantages of the Br-rich material over the calcified cuticle were a nine times greater energy of fracture and an absence of large scale inclusions that might limit sharpness and concentrate stresses. Here we propose to use these instruments and techniques in order to compare the different types of HEBs to each other and to man-made materials. We will compare previously unmeasured properties of Zn- and Mn-enriched regions in scorpion stings, Zn- and Fe-enriched regions in the jaws and paragnaths of nereid worms, and the Zn- and Mn-enriched regions in spider fangs and teeth. We propose to add impact fracture resistance to our suite of measured properties because we suspect that the heavy elements increase damping of high frequency vibrations from impacts by virtue of their mass density (Schofield *et al*., 2009). We hypothesize that smaller organisms trade hardness and modulus of elasticity for fracture resistance and the ability to make sharp structures. We argue that this is because of strength limitations and scaling rules.

In addition to an incomplete knowledge of the mechanical properties of HEBs, the binding chemistry is not understood. The heavy elements do not appear to be bound in biomineral form, but, especially for Zn-rich materials, there is too much to be bound as Zn^{+2} to proteins. One possibility is that the Zn is present in biomineral nano-clusters. In our previous NSF project we tested this hypothesis by looking for Zn-Zn scattering in X-ray Absorption Spectroscopy data (Tao *et al*., 2007) . The results ruled out most biomineral inclusions but did not rule out certain possibilities with highly variable Zn-Zn spacing. Here we will propose to look for a highly disordered nano-phase using Laser Atom Probe Tomography (APT), and other techniques. We will also use these techniques to examine Fe, Mn and Cu HEBs.

In summary, we will investigate the atoms bound to and in the vicinity of Zn, we will compare the mechanical properties and chemical compositions of several HEBs, we will test whether HEBs are particularly resistant to fracture from impact, and we will search for a correlation between tool size and the use of more fracture resistant materials.

We will use the term Heavy Element Biomaterial (HEB) to refer to any structural biological material that contains high (1% or greater) concentrations of one of the heavy elements Fe, Mn, Zn, Cu, or Br, and does not appear to have large-scale biomineral inclusions. We believe that we are justified in grouping together materials from distantly related organisms because Extended X-ray Absorption Fine Structure (EXAFS) analysis suggests that the chemical environment of particular enriching elements are similar even in distantly related organisms (Zn, Br: Tao *et al*., 2007; Fe, Mn, unpublished preliminary data).

As humans continue to build ever smaller machines, we may learn from the materials that have evolved in small organisms to meet the distinctive challenges associated with mechanical interactions on a small scale. A greater understanding of these biomaterials may lead to transformative approaches to man-made materials in the sense that these biomaterials may precisely control balances of mechanical properties by controlling water binding, and that they may limit impact fracture by incorporating heavy elements.

This proposal is similar to a proposal submitted last year, but has been modified in response to panel suggestions: we have included a more detailed broader impact section and we have collected preliminary APT data.

BACKGROUND AND RESULTS FROM RECENT NSF SUPPORT

The recognition that the fracture-prone tools of many small organisms contain unique materials has developed slowly over the last couple of decades. The first indication of distinct materials came from Bryan and Gibbs who found Zn in the jaws of nereid marine worms and Cu in the jaws of glycerid marine worms and suggested that they might improve the mechanical properties of the jaws (Bryan and Gibbs 1979; Gibbs and Bryan 1980a,b, c). Subsequently, Hillerton and Vincent (1982) and Hillerton et al. (1984) detected Zn and Mn near the cutting edge of the mandibles of many insects (Hillerton *et al*., 1982).

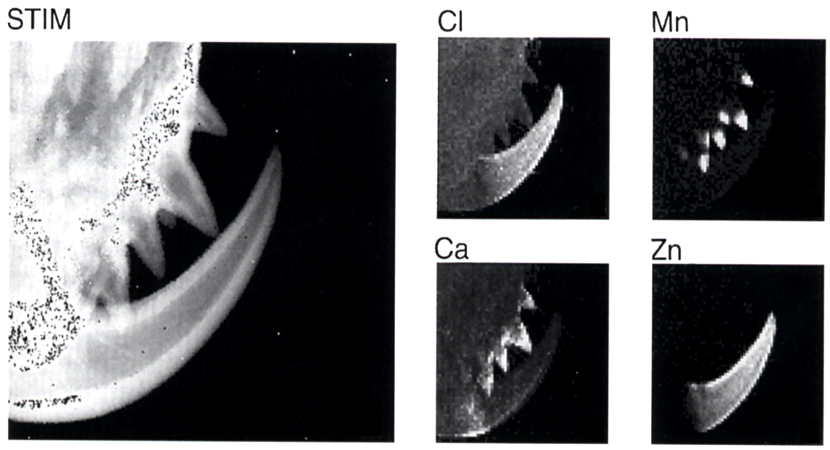

Figure 1. STIM and PIXE images showing a cheliceral fang and marginal teeth from a garden spider, *Araneus diadematus*. The large image is a STIM(Scanning Transmission Ion Microscopy) image in which lighter shades indicate greater projected mass. The four smaller images are PIXE (Proton Induced X-ray Emission) images of the same field as the STIM image showing the origin of Cl, Ca, Mn, and Zn X-rays. Lighter shades indicate greater numbers of detected X-rays. The fang is enriched with Zn and Cl. The tips of the marginal teeth contain Mn. Ca is enriched in the teeth, surrounding regions and the portion of the fang not enriched with Zn. Backrounds have not been subtracted. Frame size: 1 mm x 1mm. Source: Schofield, 2001.

Because of the spatial imprecision of the atomic absorption spectroscopy used to quantify these metals, these studies could only hint at the extreme magnitudes of the metal concentrations in localized regions. Our group stumbled into the field after developing high-energy ion microscopy (Schofield *et al*, 1988; Schofield, 1990; Schofield & Lefevre, 1992, 1993), and observing Zn-rich mandibular teeth in an ant captured while crossing the

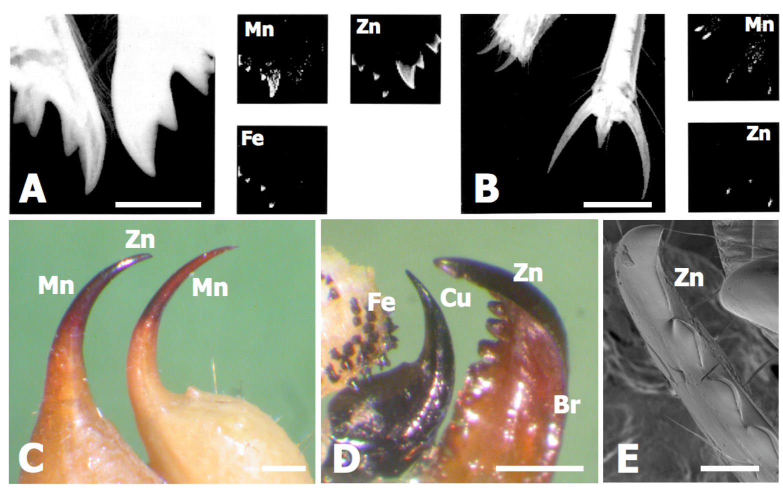

Figure 2. Images of HEB-containing tools from our collection. **A**: cheliceral tynes (mouth claws) of the scorpions *Centruroides exilicauda* (left) and *Paruroctonus mesaensis* (right). **B**: leg claws of the scorpions *Centruroides exilicauda* (left) and *Vaejovis confusus* (right). **C**: Stings of the scorpions *Vaejovis confusus* (left) and *Centruroides exilicauda* (right). **D**: tools of marine worms, paragnaths of *Nereis vexillosa* (left), jaw of *Glycera convoluta* (middle), jaw of *Nereis vexillosa* (right). **E**, mandible of the ant *Atta cephalotes*, showing the cutting edge, measured to have a 50 nm edge radius. Scale bars: 0.5 mm

lab floor. High-energy ion microscopy enabled rapid surveys of whole structures and concentration measurements on a micron scale. We found that different regions of a single animal could be enriched with different metals, such as Zn in the fangs and Mn in the marginal teeth of spiders (Figure 1) and we found that these enriched biological materials were employed in structures other than mouthparts, such as leg claws and stings (Schofield & Lefevre, 1989, Schofield *et al*., 1989). Using the high-energy ion microprobe, we found HEBs in organisms from four phyla, in the contact regions of structures such as mandibles, jaws, claws, and stings (Figure 2). Our findings, along with those of other researchers, were reviewed in Schofield (2001). These structures are used for cutting, piercing, digging, grasping, clamping and traction. In each of these structures, HEBs were localized in regions susceptible to mechanical force from direct contact with the environment. We did not find HEBs in structures such as joints that might benefit from hardening or abrasion resistance but do not directly contact the environment. The enriched structures were often sharp and susceptible to fracture, abrasion and impact. But these impressions developed over time, and we did not look for correlations between size and sharpness or between sharpness and the presence of HEBs. However, we did maintain a collection of the hundreds of organisms we examined.

Results from recent NSF sponsored research will be split into labeled sub-sections of the mechanical properties section and the composition section, while broader impacts will be discussed at the end of the proposal.

MECHANICAL PROPERTIES

Several early investigations found a higher indentation hardness for Zn-enriched structures than for surrounding regions (Hillerton and Vincent, 1982, Hillerton, Reynolds & Vincent, 1982, Schofield, 1990, Edwards *et al*., 1993, McClements *et al*., 1993). However, similar levels of hardness had also been found in certain Zn-free regions (Hillerton, Reynolds & Vincent, 1982). To strengthen the correlation between Zn-enrichment and hardness, we took advantage of our observation that Zn is incorporated after tanning, very late in cuticle development (Schofield, Nesson & Richardson, 2003; NSF grant IBN 9817206). We measured the hardness of the mandibular teeth of adult ants, using nanoindentation, during the period when Zn concentration increases in early adult life (Schofield, Nesson & Richardson, 2002; NSF grant IBN 9817206). We found that the hardness increases nearly threefold as the Zn is incorporated, from about 0.3 to 0.9 GPa.

More recently, Broomell *et al.* (2006) found that removal of Zn with EDTA from core regions of the jaws of a nereid worm reduced hardness from about 0.7 to about 0.2 GPa. Furthermore, when the depleted regions were soaked in $ZnCl_2$, $MnCl_2$ or $CuCl_2$ the hardness was substantially restored (Broomell, Zok & Waite, 2008). The EDTA, however, did not remove Zn from the more biologically relevant contact surfaces, as is also the case in the arthropods we have studied (unpublished data).

Hardness and modulus of elasticity are, almost exclusively, the only mechanical properties that have been measured for HEBs, not necessarily because of their relevance, but because existing indentation machines that measured these properties could test small specimens. Several authors have lamented the inability to measure other properties on these small samples and have attempted to construct estimates from hardness (H) and modulus of elasticity (E_r). For example, Lichtenegger *et al.* (2002) claimed that, based on high values of $H^{3/2}/E_r$, glycerid worm jaws had high abrasion resistance. Such an inference has little justification (Schofield & Nesson, 2003). For example, the model wrongly predicts the Br HEB to be 2 times as abrasion resistant (lower wear rate) as nylon, when a realistic test showed it to be only 1/3 as resistant (Schofield *et al*., 2009 & Table 1 below). Other groups have also employed this oversimplification as a proxy for actual measurements (Weaver *et al*., 2010, Politi *et. al*, 2012).

Direct measures of abrasion resistance have been attempted on HEBs using nano-indentors, which can be used to estimate wear rates in some materials by measuring the depth of pits made by repeatedly scanning the probe (Pontin *et al*., 2007). We also have tried this technique, as well as nano-indenter scratching techniques, and rejected them for HEBs because repeated wear sessions did not continue to deepen the pit, as would be expected if mass were being removed. Thus, for HEBs, the measured property was similar to indentation hardness not wear rate, probably because the forces were not high enough to abrade particles. In general, we argue for the importance of scale in measuring mechanical properties; that is, the measure of abrasion resistance for a material is most relevant when the size of the abrading particles, the forces applied and the interaction velocity are like those experienced by the material in its natural setting.

Scale is also important in comparing properties of biological materials to standard engineering materials. While most of the values from microscopic tests are comparable to values from macroscopic tests, the nano-indentation test of the modulus of elasticity of PMMA yields values of 6 or 7 GPa, while values from macroscopic tests are typically around 3 GPa (Giddings *et al*., 2001; Ishiyama & Higo, 2002). Comparisons of microscopic measurements on biomaterials to macroscopic measurements on engineering materials have led to overestimations of the advantages of biological materials (Broomell *et. al*., 2006), so we only make comparisons to engineering materials that we have actually measured with our equipment.

Mechanical property results from recent NSF support

For our most recent NSF grant (IOS 0422234) we addressed the nano-indentation issues and developed a suite of instruments and techniques to measure abrasion resistance, energy of fracture, storage and loss modulus in addition to improvements in techniques to measure hardness and modulus of elasticity (Schofield et al., 2009). Our instruments for measuring fracture toughness and abrasion resistance are based on standard testing methods, but allowed for much smaller specimens. We found that the tips of the claws of certain crab species were not calcified like the rest of the claw, but were instead enriched with Br. So we used the lined shore crab, *Pachygrapsus crassipes,* to compare the biomineralized cuticle to an HEB within a single organism (Table 1 and 2 – larger versions in Schofield *et al*., 2009).

Table 1
Quasi-static mechanical properties.

Material	Wear rate (w), mm^3/J	n	Energy of fracture (K), J/m^2				Hardness (H), GPa		Reduced modulus of elasticity (E), GPa		n	Force challenge[e] $\sqrt{(KE)}$	Displacement challenge[e] $\sqrt{(K/E)}$
			Transverse to cuticle projection[d]	n	Perpendicular to cuticle surface[d]	n	Oliver–Pharr	From image[c]	Oliver–Pharr	From image[c]			
Calcified crab cuticle (*P. crassipes*)	0.8 ± 0.2 M = 330	7	348 ± 138 M = 210	6	233 ± 65	7	1.49 ± 0.40	1.79 ± 0.35	35 ± 12	35 ± 11	38	3.4×10^6	1×10^{-4}
Bromine-rich crab cuticle (*P. crassipes*)	1.0 ± 0.2 M = 2930	7	2931 ± 574 M = 2005	8	1991 ± 551	6	0.49 ± 0.12	0.60 ± 0.08	7.6 ± 2.7	7.6 ± 2.2	45	4.7×10^6	6.2×10^{-4}
Un-enriched ant cuticle (*Atta sp.*)			3885 ± 2514 M = 3423	9	1557 ± 545 M = 1690	8	0.33 ± 0.07	0.37 ± 0.06	5.2 ± 1.6	5.0 ± 1.6	35	4.1×10^6	8.3×10^{-4}
Cat claw (keratin)			13321 ± 8810 M = 12684	9			0.31 ± 0.03	0.35 ± 0.02	5.5 ± 0.5	5.3 ± 0.3	6	8.2×10^6	15×10^{-4}
Salmon tooth			2255 ± 649 M = 2088	5			1.13 ± 0.01	1.30 ± 0.04	23 ± 1.2	23 ± 1.4	3	6.9×10^6	3.0×10^{-4}
Calcified crab spoon tip cuticle (*Pugettia producta*)							1.4 ± 0.1	1.5 ± 0.2	32 ± 3	30 ± 2	3		
Nylon 6 (not dried)	0.30	1					0.20 ± 0.01	0.14 ± 0.01	3.1 ± 0.14	2.5 ± 0.16	3		
PMMA (acrylic)							0.33 ± 0.04	0.29 ± 0.07	6.8 ± 0.8	5.9 ± 0.6	14		
Polycarbonate							0.26 ± 0.004	0.25 ± 0.004	3.2 ± 0.04	2.8 ± 0.03	3		
Fused silica							10.7 ± 0.7	12.8 ± 1.7	71 ± 8	[a]70 ± 12	[b]		

M: median, which is less sensitive to out-lying samples that may not have fractured quasi-statically.
n: number of specimens.
[a] This value was set to 70 GPa by adjusting the calibration factor for the area of the indents. This calibration factor is used to account for systematic errors in measuring the area of the indentation using SPM images. This area calibration factor was used in calculating all other image-based hardness and modulus of elasticity values. The Oliver–Pharr measurements were not adjusted to give a particular value, instead, they are derived from ESEM measurements of the indenting tip shape and the factory calibration of the force and displacement of the indenting transducer. The fact that the resulting value, 71 GPa, for fused silica is close to the accepted value of 70 GPa, is evidence that the factory instrument calibration is correct.
[b] n = 14 for values from images and n = 28 for Oliver–Pharr values.
[c] Areas measured on AFM images of the residual indentations were used in the calculation.
[d] For fracture orientations, see Fig. 11.
[e] For "K", the transverse median value was used, for "E", the image value was used.

R.M.S. Schofield et al. / Journal of Structural Biology 166 (2009) 272–287

Table 2
Dynamic mechanical properties.

Specimen	Storage modulus (GPa)		Loss modulus (GPa)		tan(δ) (relative to silica)		Number of specimens
	1 Hz	10 Hz	1 Hz	10 Hz	1 Hz	10 Hz	
Calcified crab (*P. crassipes*) cuticle	36 ± 10	42 ± 10	2.1 ± 1.3	0.79 ± 0.65	0.06 ± 0.02	0.02 ± 0.02	20
Bromine-rich crab (*P. crassipes*) cuticle	7 ± 3	8 ± 2	0.8 ± 0.3	1 ± 0.3	0.12 ± 0.06	0.13 ± 0.07	21 (1 Hz) 19 (10 Hz)
Plexiglass (acrylic)	5.9 ± 0.03	6.7 ± 0.2	0.531 ± 0.006	0.73 ± 0.07	0.090 ± 0.001	0.108 ± 0.008	3
Nylon 6 (not dried)	3.16 ± 0.06	3.6 ± 0.2	0.27 ± 0.01	0.37 ± 0.02	0.086 ± 0.002	0.10 ± 0.01	3
polycarbonate	3.58 ± 0.06	3.86 ± 0.02	0.09 ± 0.02	0.23 ± 0.01	0.026 ± 0.004	0.060 ± 0.003	3
PMMA	6.6 ± 0.8		0.75 ± 0.27		0.11 ± 0.04		17

p for Wilcoxon two-sample test for tan(δ): 1 Hz Ca vs Br: $p = 1.3 \times 10^{-4}$; 10 Hz Ca vs Br: $p = 1.8 \times 10^{-7}$.

Our results suggest that the main mechanical advantage of the Br HEB over the calcified cuticle is resistance to fracture. Conclusions reached from comparing the mechanical and structural properties of these materials are summarized below in list form. Chemical properties will be discussed in the composition section.

1) The Br-rich claw cuticle was similar to calcified cuticle in resistance to our abrasion test; it was ~ 1/3 as hard as the calcified cuticle, and its modulus of elasticity was ~1/5 as great. On the other hand, the energy required to produce a fracture of unit area was ~9 times greater for the Br HEB than for the calcified material. Relative to non-enriched cuticle from ant mandibles, the Br-enriched cuticle was about equal in energy of fracture, but was ~1.5 times as hard and had a 1.5 times greater modulus of elasticity.
2) For sinusoidal indentations at 10 Hz, we found that the rate of energy loss was several times greater for brominated cuticle than for calcified cuticle. Eighty-six percent of the energy stored in vibrating structures of the Br HEB would be lost in only 2.5 cycles. While we did not directly test dynamic mechanical properties at the much higher frequencies associated with impact, we did demonstrate qualitatively that the brominated cuticle was more resistant to impacts from bead blasting.
3) The greater viscoelasticity and plasticity of the Br HEB that we observed should further enhance fracture resistance because some energy is diverted into rearranging molecules. Because of the viscoelasticity, the Br HEB can withstand a greater deformation before fracture for slower interactions.
4) Our results suggested that the main advantage of brominated cuticle over calcified cuticle was resistance to fracture. Other things being equal, a spoon tip made of brominated instead of calcified cuticle would require a 6 times greater deflection, and a 1.4 to 3 times greater force to produce the same area of fracture. The main advantage of brominated cuticle over unenriched cuticle was that it had a higher modulus of elasticity and hardness (though not as high as for calcified cuticle). Our data suggested that calcified cuticle would be best for regions designed to apply large pressures, but that were not sensitive to fracture, and that the brominated cuticle would be optimal for sharp edges and tips that must keep their shape under pressure and not fracture.
5) A comparison with other natural and man-made materials indicated that the Br HEB was harder and stiffer than the hardest tested plastic, acrylic (PMMA). Unenriched mandible cuticle was about the same hardness as acrylic. Brominated cuticle was comparable to acrylic in low frequency dynamic mechanical properties. Salmon tooth was harder but less fracture resistant than brominated cuticle, and cat claw was more fracture resistant but not as hard.
6) The largest structures observed in the Br HEB were 7 nm laminae, 1/1000 of the size of crystals in calcified cuticle, making possible tips or edges that were sharp on a micron scale and smaller.
7) We hypothesized that smaller organisms would be more likely to trade hardness and stiffness for fracture resistance, based on the importance of fracture resistance in regions of small cross sectional area. We hypothesized that small organisms will tend to employ sharp tips and edges slowly, when possible, in order to take advantage of viscoelasticity and to reduce uncontrolled forces that can lead to fracture.
8) We also speculated that heavy elements employed in HEBs may improve absorption of energy from impacts by virtue of their high mass density, lowering the resonant frequencies of low-frequency molecular motions to give more overlap with the range of vibration frequencies produced by impacts.

Wear matters
A second part of our most recent NSF grant, IOS 0422234, was to investigate the degree to which average levels of wear influenced the performance of arthropod tools. We used leaf cutter ants (*Atta* sp.) as a model and found the cost of wear to be greater even than we expected (Schofield *et al.*, 2011). We measured the cutting rates of leaf cutter ants in Panama and then collected the cutting ants to measure wear of their mandibles. We also developed an instrument to measure the force required to cut leaves using their dissected mandibles. From our measurements of cutting rate and cutting energy as a function of wear, we estimated that a hypothetical colony of leaf cutter ants with un-worn mandibles would have spent about half as much time and half as much energy cutting leaves outside the nest than our field colony did. Fac-

tors of two in energy can be expected to produce huge evolutionary pressures and we were not surprised to find that the ants with the most worn mandibles carried leaves but did not cut them.

We found that *Atta* leaf cutter ant mandibles start out literally as sharp as a razor blade (50 nm blade radius), and that the force required to cut leaves is about what it would be for a pristine scalpel blade.

COMPOSITION

The concentrations of heavy elements in HEBs are difficult to reconcile with standard binding mechanisms, either in biominerals or to proteins. The problem with binding in a biomineral can be illustrated by a comparison to the volume fraction of goethite (FeO(OH)) in the radular teeth of limpets, which is roughly 0.5. In contrast, if Zn were incorporated as ZnO (Zn minerals containing Al, Si, P, and S have been excluded by elemental analysis and $ZnCO_3$ has been excluded by EXAFS), then the mineral would occupy less than 0.07 of the volume. This is likely to be too little of the putative Zn biomineral to fill the tissue to the extent that the mechanical properties of the tissue would approach those of the pure mineral.

On the other hand, the 25% Zn concentration in scorpion sting cuticle is too high to be accounted for by binding of individual ions to proteins. Hillerton and Vincent (1982) presented such a hypothesis, suggesting that Zn in the mandibles of insects might increase the number of secondary bonds in the cuticle and thereby increase the density and fracture toughness of these structures. Amino acid analysis of the tips of worm jaws indicate elevated fractions of histidine, which has led to the suggestion that Zn cross-links proteins as $Zn(His)_3$ Cl (Bromell *et al*., 2006, Birkedal *et al*., 2006, Broomell *et al*., 2008).

Broomell *et al*. (2008) sequenced the dominant protein from the Zn-enriched jaws of a nereid worm, and found no well-defined repetitive pattern. About 27% of the amino acids were histidine. If Zn were bound in $Zn(His)_3$ Cl, the maximum concentration would be about 5% (in contrast, we have measured concentrations of about 18% in nereids) and this analysis does not consider the problem of orienting every histidine so that it can participate in a Zn-mediated cross-link at the proper angle. It is clear that only a minor fraction of Zn atoms could be involved in cross-linking proteins. If, on the other hand, only one histidine were bound to each Zn atom, then the Zn concentration would be about 14% if the jaw tip were entirely made of this protein, so even a model in which each Zn^{2+} is attached to only a single histidine instead of three, does not account for the full Zn content.

Composition results from recent NSF support

Zinc HEB

For IOS 0422234, we tested the hypothesis that Zn was bound in nano-scale biomineral inclusions using X-ray absorption spectroscopy (XAS) and other techniques. Our XAS results did not support this hypothesis; we found no Zn – Zn scattering signal when we would expect to find such a signal even for amorphous biominerals (Tao *et al*., 2007). These results suggest that either the Zn atoms are further apart than about 0.6 nm, or else that the Zn-Zn distance is highly variable. In addition, we found no signs of order in X-ray and electron diffraction experiments and no distinguishable biomineral phases were evident at a TEM resolution of about 5 nm. These results, which included spider fangs, also support the interpretation that ZnO found in spider fang preparations was artifactual (Politi *et. al*, 2012) .

EXAFS did reveal the similarity of the chemical environment of Zn across many organisms from multiple phyla (Figure 3). For each of the organisms examined, EXAFS indicated that Zn is 4-coordinate, bound to O and/or N, and, for arthropod specimens, the data suggests that only one in four of these binding sites could be an imidazole, while two imidazoles could be possible in the worm jaws. EXFAS data are also consistent with binding to the oxygens and/or nitrogens of chitosan (Braier & Jishi, 2000, Okuyama *et al*., 1997, Schlick, 1986), or catechols (unpublished analysis). EXAFS data are not consistent with Cl binding as proposed by Broomell *et al*. (2006, 2008). We have recently noticed that these EXAFS spectra are very similar to spectra for Zn in concentrated aqueous hydroxide (Pandya *et. al*., 1995).

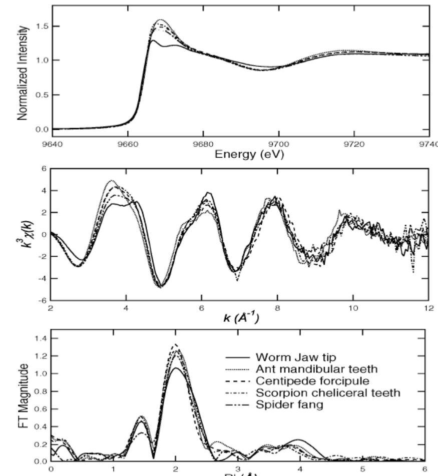

Figure 3. Comparison of Zn XAS data for a worm, an ant, a centipede, a scorpion and a spider. In all cases the chemical environment appears similar.

Bromine HEB

In our previous NSF research we studied a Br HEB used by a crab. Using X-ray absorption spectroscopy we found that the Br is most likely bound in singly brominated phenyl rings (Schofield *et al*., 2009). The Br EXAFS spectra of spoon tips were similar to spectra for models and standards of brominated phenyl rings, were less similar to spectra for brominated histidine, and dibrominated phenyl rings, and were quite different from Br EXAFS of an aliphatic Br compound. The percentage of amino acids that were tyrosine (which contains a phenyl ring) was about 10 times greater in the claw tips (about 5%) than in the calcified region. There were twice as many tyrosine residues as required to bind all Br atoms.

The Br EXAFS spectra for *P. crassipes* spoon tips were similar to spectra from the tarsal claw tips of another shore crab species, and similar to the spectra from Br-enriched mechanical structures of several other invertebrate phyla: nereid polychaetes, a pycnogonid, and a priapulid. EXAFS of the Br in the nereid jaws was consistent with singly brominated tyrosine, but not multiply halogenated tyrosines observed elsewhere (Birkedal *et al*., 2006). Our results, together with previous results, indicate that many invertebrates employ singly brominated phenyl rings in mechanical structures.

PROPOSED RESEARCH

Our proposed research is guided by an over-arching hypothesis for the function and form of HEBs. We think that HEBs are employed because they are fracture resistant (especially to fracture from impact) for materials with their levels of hardness and modulus of elasticity (fracture resistant materials are usually softer). We think that multiple HEBs are often employed for their different balances of fracture resistance and hardness/modulus of elasticity. We speculate that HEBs 1) increase resistance to impact fracture by increasing absorption of high frequency vibrations, 2) increase resistance to fracture and enable sharp structures by being homogeneous on a smaller scale than the rest of the cuticle so that there are no inclusions or notches that concentrate stress or limit sharpness, 3) control hardness and modulus of elasticity by precisely controlling the tightness of water binding and the quantity of water (the idea is that water enables slippage between proteins, and the binding or the replacement of water decreases this effect, reducing plasticity and increasing hardness). We suspect that the dominant heavy elements are mainly important in their mass densities and that the particular element is possibly determined by the metal- or halogen-binding protein from which the material evolved. Our proposed research is organized around testing four hypotheses, two related to mechanical properties and two related to composition: **I. HEBs have high resistance to fracture from impact for their levels of hardness, and different HEBs have balances of**

properties consistent with their locations in tools; II. smaller organisms tend to employ sharper tools, which tend to employ HEBs; III. Zn is bound to three hydroxides and either a fourth hydroxide or a histidine imidazole nitrogen, in nanometer-scale inclusions. Fe, Mn, and Cu HEBs also do not contain inclusions larger than 10 nm; IV. the hardness of HEBs is inversely correlated with loosely bound water content.

MECHANICAL PROPERTIES

With the instruments and techniques developed for our previous grant, we finally have the tools to investigate the differences in mechanical properties between the various HEBs. In addition, while it is not exactly a mechanical property, the ability to make sharp structures is, we believe, an important advantage of HEBs for small organisms. Thus we will also test our hypothesis that smaller organisms use HEBs to make sharper tools.

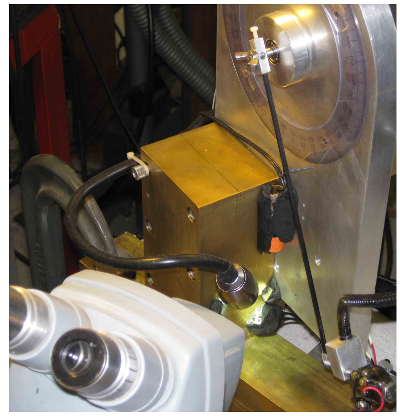

Figure 4. Pendulum-based impact tester. The carbon fiber pendulum is mounted on precision bearings, released electronically and has a flat top conical diamond tip. A laser/ photodiode pair monitors pendulum motion. Fracture is monitored using the microscope.

A note on sample preparation: we are convinced that mechanical properties of organisms should be studied in as close to natural conditions as possible (Schofield & Nesson, 2003) – this is particularly important because of the role of water in setting properties. The organisms will be kept alive until just before testing. We have extensive experience housing these organisms in our lab in nature-like conditions (e.g. similar relative humidities).

I. HEBs have high resistance to fracture (especially from impact) for their levels of hardness/modulus of elasticity. Different HEBs have different balances of hardness and energy of fracture that are consistent with their locations in tools (e.g. Zn materials are always harder but less fracture resistant than Mn).

For Zn, Mn and Fe HEBs we will measure the suite of properties that we measured for crab materials (Schofield et al., 2009): hardness, modulus of elasticity, wear rate, energy of fracture, and storage and loss modulus. In addition, we will measure resistance to fracture from impact using a machine that we have prototyped. We will compare impact fracture resistance for Zn, Mn and Fe HEBs to un-enriched cuticle and hard plastics. We expect to find that impact fracture resistance is greater for HEBs than for other materials of equal or even of somewhat lower hardness, even though harder materials are typically more fracture prone (Vogel, 2003).

To demonstrate that we can measure the impact response of small tools, we built a prototype impact testing machine (Figure 4) that is pendulum-based but differs from a Charpy test in that the specimen is not broken but the energy to produce a surface fracture using a 150 um diameter pendulum tip is measured (we do not necessarily consider this to be the final test protocol). We found that Zn-rich regions of the nereid jaws required 1.7 times as much energy to produce a surface fracture as adjacent Zn-free regions (see Table 3). This preliminary result is particularly interesting because the Zn-rich material is more resistant to impact fracture than the Zn-free material. In contrast, the quasi-static energy of fracture in ant mandibles is much lower for the Zn-rich material than the Zn-free material (Table 3). These preliminary results do seem to support our speculation that the heavy element material is particularly resistant to impact fracture because it helps absorb the high-frequency energy from impact (Schofield et al., 2009, Boyd, 1985a,b; Wert, 1986).

We will continue to use our strategy of comparing two materials within a single organism to reduce inter-organismal differences that may distort the comparisons. We will measure our suite of properties for: Zn

Table 3. Preliminary results for mechanical properties

Material	Wear Rate (mm^3/J)	n	Energy of Fracture (J/m^2)				Hardness (Gpa)	Reduced modulus of Elasticity (Gpa)	n	Impact resistance, 150 um tip (mJ)	n
			Transverse to cuticle projection	n	Perpendicular to cuticle surface	n					
Ant, Zn-free region (*Atta sp.*)			3885 $\pm$ 2514	9	1557 $\pm$ 545	8	0.37 $\pm$ 0.06	5.0 $\pm$ 1.6	35		
Ant, Zn region			546 $\pm$ 151	4			0.98 $\pm$ 0.7	6.5 $\pm$ 1.3	21		
Spider fang, Zn region (*A. dia.*)	0.7 $\pm$ 0.3	5	925 $\pm$ 695	5							
Worm jaw, Zn-free region										0.45 $\pm$ 0.22	8
Worm jaw, Zn region (*N. bra.*)							0.67 $\pm$ 0.04	10.2 $\pm$ 0.5	4	0.75 $\pm$ 0.23	8
Scorpion sting, HEB-free reg.							0.53 $\pm$ 0.05		6		
Sting, Zn region, (*H. Ariz.*)							1.74 $\pm$ 0.21		19		
Sting, Mn region, (*H. Ariz.)*							1.15 $\pm$ 0.27		22		
Scorpion chelicera, Zn & Fe region, (*C. exilicauda*)							1.38 $\pm$ 0.37		8		
Chiton radula, Fe biomineral							8.6 $\pm$ 1.7	84 $\pm$ 15	13		

and Mn regions of the sting of a scorpion *Hadrurus arizonensis*, and Zn and Fe regions of the jaws and paragnaths of the worm *Nereis brandti.* These species have been selected to meet the shape and size requirements for the most difficult of the tests. To further test the hypothesis that these materials are similar in different species we will test selected properties for the Zn and Mn regions in the fang and marginal teeth of the spider *Araneus diadematus*, and the Fe and Zn regions of the chelicera of the scorpion *Centruroides exilicauda*.

Our hypothesis that Mn HEBs are more fracture resistant than Zn HEBs, but are not as hard, is based on the locations of these materials in biological tools. There is a common pattern of Mn in the shafts and Zn in the tips of the tools of very different organisms (see Figure 5). The shaft must be stiff, but can trade hardness for the ability to resist fracture (in particular, the shaft should have a high product of energy of fracture and modulus of elasticity). Our preliminary hardness measurements on scorpion stings (Table 3) support this hypothesis: the Mn region is not as hard as the Zn region. We predict that the Mn HEB will have a higher product of modulus of elasticity and energy of fracture than either the Zn HEB or the normal cuticle.

Also, a material that is intermediate in properties between Zn-enriched and unenriched cuticle may reduce the chance that the Zn-rich tip will break off at the interface. We hypothesize that the Mn-rich material has an intermediate modulus of elasticity between the Zn-rich material and un-enriched material, reducing the likelihood of fractures at the interface of the Zn material.

Finally, preliminary results in Table 3 show that the scorpion Fe-rich material has properties more like HEBs than the Fe biomineral in the chiton radula, as expected.

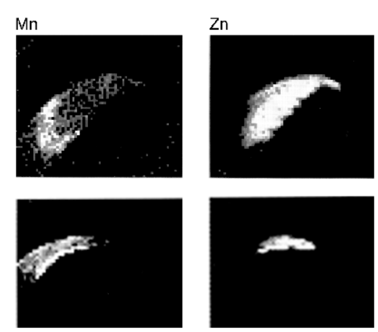

Figure 5. The distribution of Mn (left column) and Zn (right column) in jaws of the worm *Nereis virens* (top row) and the sting of the scorpion *Vaejovis confusus* (bottom row). Zn is located in the tips and Mn in the shafts in these tools from 2 different phyla.

II. Smaller organisms tend to employ sharper tools, which tend to employ fracture resistant materials such as HEBs

Smaller organisms often must cut or puncture the same materials as larger organisms, e.g. a mosquito and a lion must both puncture the same skin, a sloth and a leaf cutter ant must cut the same leaves. Sharper tools make it possible for smaller organisms to concentrate their lesser forces to reach the necessary stresses to break bonds in these membranes. But we argue that tools with smaller cross sectional area need to have higher energies of fracture. Our proposed scaling rule is based on the assumption that the risk of punch fracture is related to the ratio of the energy required to penetrate the membrane over the energy required to fracture the punch. This ratio goes as 1/r (r is the punch radius) because only membrane bonds around the perimeter of the punch need to be broken to penetrate the membrane, while all bonds in a cross section of the punch need to break to fracture the punch in two. Thus to maintain an equal risk of fracture, the energy of fracture must be proportional to 1/r. This scaling rule also applies to cutting blades, because the fracture is unlikely to be much longer than it is wide. Man-made cutting instruments may also be force-limited or require very sharp tips for other reasons, and may thus require special fracture-resistant materials because of this scaling rule.

The danger of fracture for smaller organisms is further exacerbated by the need for high-aspect ratio tools (see Figure 2). This is because the tool must have a smaller radius to compensate for lower maximum forces, but it must still be long enough to penetrate to the necessary depth.

We propose to look for correlations between organism size and the radius, aspect ratio, and composition of tool tips. This will reveal which of the HEBs (or other materials) are used in the sharpest structures. It may be that the Mn HEB is used in the sharpest structures because it is more fracture resistant though not as hard as the Zn HEB (see preliminary mechanical property results below).

To measure tool sharpness we will use SEM techniques similar to those we used to measure the sharpness of ant mandibles (Schofield *et al*., 2011), as well as FIB/SEM profiling or TEM measurements of cross-sections when necessary. In addition to tip or edge radii, we will estimate aspect ratio using a figure of merit such as the tool radius at a distance of 10 tip radii from the tip. We will compare tools that are primarily used to penetrate or cut, from our collection of tools from hundreds of organisms in several phyla (e.g. Fig. 2). These tools were examined using ion microscopy in our phylogenetic surveys of HEBs (Schofield, 2001). The tools were originally selected before the presence of heavy-element materials was determined, reducing the chance of bias. In addition to typically worn tools, we will also measure the sharpness of pristine unused tools where possible.

We will augment the size range of organisms in our collections using museum collections and literature values (e.g. Evans *et al*., 2005). In order to reduce the chance of bias, our museum sampling technique will be informed by similar studies (e.g. long bone scaling; Christiansen, 1999). We will look for correlations between body length and tip or edge radius normalized by body length, to investigate whether radii decrease more rapidly than would be expected from isometry. At least some smaller organisms use sharper tools even when sharpness is scaled by body length. If we scale up the 50 nm sharpness of ant mandibles (Schofield *et al*., 2011) by a body length ratio of 100, we would expect 5 μm blades, almost two orders of magnitude smaller than observed for mammals of that size (Evans *et al*., 2005).

COMPOSITION

III. Zinc is bound to three hydroxides and either a fourth hydroxide or a histidine imidazole nitrogen, in nanometer-scale inclusions. Fe, Mn, and Cu HEBs also do not contain inclusions larger than 10 nm

Our prior EXAFS work did not detect second shell Zn. This rules out ordered biomineral inclusions, and even amorphous materials with an approximately regular Zn-Zn distance. However, if the Zn-Zn distance is highly variable, there may be no Zn-Zn EXAFS peak. This is the case with supersaturated aqueous Zn hydroxide solutions, which have very similar EXAFS spectra to those from Zn HEBs (Pandya *et. al*., 1995, Debiemme-Chouvy, 1995). The possibility that Zn is bound in nano-scale inclusions of aqueous Zn hydroxide can be investigated with Laser APT and TOF-SIMS, which can directly determine the atoms surrounding or bound to Zn, respectively. We will also use ^{67}Zn NMR in an attempt to distinguish between Zn binding to nitrogen and oxygen.

Cross-linking does not play an important role in modulating the mechanical properties of the Zn HEBs according to this hypothesis. Instead, the Zn hydroxide inclusions would mitigate the softening effects of water by binding and replacing water and improve energy absorption by virtue of the high density of Zn atoms; Zn would lower the resonant frequencies of proteins when bound to histidine and would absorb energy by relative motion of the high density Zn in the aqueous solution. In some sense the Zn would make the water between macromolecules a more viscous damping pot for their high frequency vibrations.

Atom Probe Tomography (APT)

Atom Probe Tomography combines a time of flight mass spectrometer with a point projection microscope capable of atomic-scale imaging of materials, with sub-nanometer (<0.2 nm) spatial resolution, in three dimensions and with ppm composition sensitivity (Kelly & Miller, 2007). APT was originally only possible with conducting materials, but recent implementation of a femtosecond ultraviolet laser pulse, to assist in field evaporation of materials with low conductivity, has permitted the high-resolution analysis of dielectric and semiconducting materials in addition to metals and alloys (Bunton et al., 2010; Devaraj et al., 2013). APT is thus the ideal tool for testing our hypothesis that Zn is located in nano-scale amorphous inclusions, and also promises to provide evidence of the binding sites by revealing which atoms are correlated with the Zn inclusions. To do this, we have initiated a collaboration with scientists at the Environmental Molecular Sciences Laboratory (EMSL) at the Pacific Northwest National Laboratory (PNNL). The Cameca 4000XHR Local electrode atom probe at EMSL is the latest generation commercially available APT instrument.

Sample preparation is particularly challenging in APT because the sample must be milled into a needle shape with a nanometer-scale tip. Biological samples similar to ours have recently been analyzed with APT, including chiton teeth, apatites, bone type mineralized tissues and even mammalian cells (Gordon et al., 2012; Gordon & Joester, 2011; Narayan et al., 2012). Nevertheless, at the suggestion of last year's review panel, the EMSL user facility has graciously, and without compensation, provided us with preliminary APT data, in order to demonstrate the feasibility of this analysis for our specimens.

Figure 6 demonstrates the preparation process for a sample from the tip of a Zn-rich tooth of a leaf-cutter ant. A focused ion beam is used to cut out and mill the sample into a needle shape. We found that the HEB material was ideal for the technique and multiple needles were made in the first attempt with no problems.

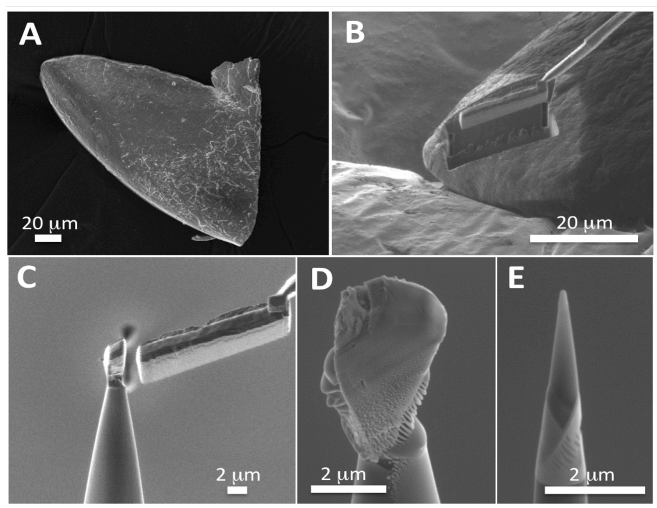

Figure 6. Preparation of an APT sample from an ant tooth. **A**: A single distal tooth from an in nest generalist-sized leaf cutter ant, *Atta cephalotes*. **B**: A nano-probe lifting out the FIB-cut specimen from the tip of the ant tooth. **C**: The end of the sample is affixed to a Si-microtip and then cut off with FIB. **D & E**: Stages in the further FIB-milling of a section to produce a tip with a final sharpness that was under 50 nm.

The APT reconstruction, highlighting the distribution of C, N, H, O and Zn, is shown in figure 7. The 2-D Zn distribution map shows ~3 nm-scale regions that are especially rich in Zn, consistent with our nano-composite hypothesis. The 3-D map shows that the Zn-rich regions are not in fiber patterns, and thus probably not associated with chitin fibrils. The detected atomic percentages do not necessarily reflect the atomic percentages in the sample. In order to obtain better estimates of the atomic percentages in the samples, we will 1) vary the laser pulse energy over the range of 0.02.pJ to

450 pJ to observe the effect on detected atomic percentages, 2) examine an ant tooth prior to Zn deposition (which happens early in adult life; Schofield, Nesson & Richardson, 2002), 3) examine standards of hypothesized Zn-compounds (e.g. $Zn(OH)_2$), 4) examine the sample needles with element-quantifying TEM techniques prior to APT analysis, and 5) undertake an amino acid analysis of the material in ant teeth, as we have done by pooling small specimens in the past (Schofield *et al*., 2009), and from this calculate atomic percentages for the proteins in the samples to compare to APT values. We will also use correlation techniques to study the binding of Zn (e.g. what atom is most likely to be found within 0.2 nm of Zn in high concentration regions, and does this differ in lower concentration regions). And we will use our Spartan modeling software to compare hypothesized distributions of atoms to the distributions in APT data.

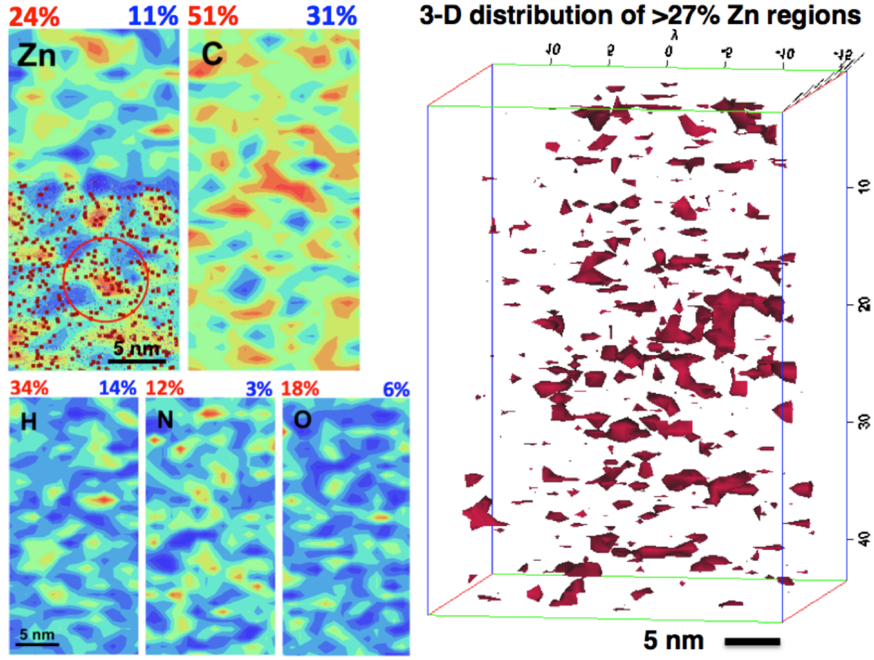

Figure 7. Preliminary APT analysis of the ant tooth specimen of Figure 6. The Zn, C, H, N, and O maps show the distribution of detected ions for a slice of the tomographic APT data. The red and blue percentages at the top of each map give the atomic percentages for the respective colors on the map (percentages of detected atoms, not necessarily the percentages in the sample). The lower half of the Zn map also shows the specific origins of Zn atoms (only 20% of the detected Zn atoms are shown for clarity). The 3-D distribution map at the right shows that the high-Zn regions do not appear to be in fiber patterns, as would be expected if the Zn were associated with chitin fibrils.

In addition, we will examine other specimens from this tooth in order to evaluate the uniformity of the material. Finally, we will extend our investigations to other HEBs, such as the Mn-rich material used by scorpions, the iron-rich material used by nereid worms, and the copper rich material used by glycerid worms.

This preliminary APT data confirms our previous results indicating that HEBs are very different from biomineralized tissues in that the Zn-rich inclusions are orders of magnitude smaller than the mineral inclusions in biomineralized tissue.

TOF-SIMS

Time-of-flight secondary ion mass spectrometery (TOF-SIMS) uses a pulsed beam of ions (Bi_1 or Bi_3 in our case) to bombard a sample, accelerating the resulting fragments over a short distance and allowing them to coast over a long distance for high resolution mass spectrometry. Schofield and Nesson are operators of the TOF-SIMS machine in the shared user facility at the University of Oregon.

TOF-SIMS analysis of organic materials is particularly difficult because there are so many different potential fragments, many of which overlap in mass, notwithstanding the high mass resolution. Fortunately, a Zn-containing fragment is expected to have peaks for each of the isotopes (3 major isotopes) in the appropriate ratios. We also paint on calibration standards (e.g. KI solution), to take full advantage of the high mass resolution. Finally, we have begun developing GSIMS – like methods for discriminating between original and recombined fragments (Gilmore & Seah, 2000, 2004, Belykh *et al*., 1997). We bombard the samples separately with Bi_1 and Bi_3, and compare the spectra, discounting those fragments with very different yields for the two ions, because of the potential of recombination. We have gathered preliminary TOF-SIMS data for Zn HEBs, and the observed fragments are consistent with our binding hypothesis: ZnNC and $Zn(OH)_3$.

^{67}Zn NMR

It may be possible to discriminate between O and N binding, which was not possible with our previous EXAFS analysis, using solid-state ^{67}Zn NMR (e.g. 3N+1O, 2N+2O, vs 1N+3O). With reasonable volumes of pooled samples, containing several percent Zn on average, our collaborators believe that, even though only 4% of the Zn is ^{67}Zn, an NMR spectrum can be acquired. Performing the NMR experiments at cryogenic temperatures combined with cross polarization (Pines, Gibby & Waugh, 1973) and spin echo techniques (Garroway, 1977) afford the needed sensitivity to overcome the low abundance (Lipton, Sears & Ellis, 2001, Lipton *et al*., 2004). Based on isotropic chemical shift and analysis of the powder line shape we should be able to make this O/N discrimination. It may be possible to go beyond this, modeling the coordination geometry and predicting the NMR parameters. This would reveal protonation states of ligands and possibly numbers of bound waters versus some other oxygen source (Lipton, Heck & Ellis, 2004).

Fe, Mn, and Cu HEBs do not contain inclusions larger than 10nm

We will examine HEBs that employ elements other than Zn using TEM, Laser APT and TOF_SIMS. While we do not expect to completely unravel the chemistry of these other systems, we believe that we can at least expect to answer the question of whether they are composed of separate phases and on what scale. We suspect that these materials are homogenous on a scale that is at least an order of magnitude smaller than the sharpness of enriched structures, such as the the 50 nm radius of ant blades (Schofield *et al*, 2011). Thus we would expect to find ~3 nm chitin fibrils, but not ~100 nm chitin fibers.

We will examine samples from the following animal models using TEM to search for large scale phases and TOF-SIMS and Laser APT to observe nanometer scale phases: 1) Zn and Mn HEBs from different regions of the stings of the scorpions *Hadrurus arizonensis* or *Paruroctonus boreus*, 2) Zn and Fe HEBs from the cheliceral tynes of the scorpion *Centruroides exilicauda*, 3) Zn and Fe HEBs from the jaws and paragnaths of the worms *Nereis brandti* or *Nereis vexillosa*. We will also examine other specimens to investigate the generality of the chemical environments, possibly including Cu from the worm *Glycera convoluta,* Zn from the mandibles of the ant *Atta cephalotes,* and Zn from the stylet of a nemertian worm (to sample a third phylum). We either have laboratory colonies or can collect these organisms, though in the past we have also had live organisms supplied by Hatari Invertebrates (Portal, Arizona).

TEM

Initial examination of suspected novel HEBs will be performed by TEM observation of ultrathin sections prepared either by ultramicrotomy or FIB/SEM lift-out techniques. Electron diffraction, energy-dispersive X-ray analysis, and STEM imaging can identify those structures which contain matrix-bound biomineral phases and at least provide an upper bound for the dimensions of possible heavy element clusters. We have access to an FEI Titan G80-200 recently installed at the EM Facility at OSU. EELS analysis with this instrument can also provide information on the valence state of Mn, Fe and Cu in HEBs.

IV. Hardness is inversely correlated with loosely bound water content in HEBs

We hypothesize that HEBs control hardness by binding and replacing water, reducing the content of loosely bound water. Water tends to soften materials, but we and others have found that Zn-rich regions are much less susceptible to softening following soaking in water than Zn-free regions. Also, when Zn is extracted from the core of nereid jaws, they become much more susceptible to softening by water (Broomell *et al*., 2008). These observations suggest that Zn acts by excluding or binding water so that it does not increases slippage between matrix macromolecules. We propose to test the hypothesis that the hardnesses of HEBs correlate with loosely bound water content using thermogravimetric analysis (TGA). TGA measures the change in weight (due to loss of water) of the specimens as temperature is slowly increased. More tightly bound water will require higher temperatures. We plan to compare the water content of un-enriched scorpion sting cuticle to Zn- and Mn-enriched regions of sting cuticle of the large scorpion *Hadrurus arizonensis*. We will make similar measurements on the jaws and paragnaths of the marine polychaetes, *Nereis brandti* (Zn & Fe) and *Glycera convoluta* (Cu), as well as the spoon tips of the crab *Pachygrapsus crassipes* (Br). We hypothesize that we will find a correlation between the percentage of mass that is lost, for example, between 50 and 150 ^{o}C, and the hardness of the 5 types of HEBs. We have lab colonies or have collected all of these species in the past and will use fresh specimens. For most of the species, we have examined the 3-dimensional spatial distribution of the HEBs (e.g. Schofield & Lefevre, 1992), and will be able to section them into regions containing only the HEBs. Schofield is experienced at use, maintenance and repair of TGA devices in the user facility at the University of Oregon, and we have made preliminary runs with a Zn HEB.

BROADER IMPACT

Humans may learn from the designs and the materials that have evolved in small organisms to meet the distinctive challenges associated with small-scale mechanical interactions. For example, similar materials may find application in sharp tools such as medical devices or atomic force microscopy tips, or in other applications that require hard but impact resistant materials.

In the sense that small organisms may precisely control balances of mechanical properties in their tools by controlling water binding, and that they may limit impact fracture using heavy elements, these materials may lead to transformative approaches to man-made materials. This research may also have application in agricultural pest control. The process by which large quantities of heavy elements are loaded into the biogenic matrices may have other material or chemical applications. For biologists, awareness of our results and scaling rules may promote recognition of behavioral and structural adaptations that mitigate fracture and wear.

RESEARCH INFRASTRUCTURE

This research is particularly interdisciplinary, combining material science with organismal biology and biochemistry, and involves collaborations with several institutions. Interdisciplinary collaborations increase the flow of ideas and technology between the fields. The continued improvement of the testing instruments we developed for our previous grant and the proposed development of an impact tester for microscopic specimens may prompt similar measurements in other systems.

RESEARCH TRAINING

We will continue our research training with a focus on under-represented groups and non-traditional students. We will continue to partner with Lane Community College, in our area, which has significantly higher fractions of minority students than U of O, especially Hispanic and Native Americans/Alaskans as well as first generation students. In our previous grant period, three students worked in the lab through the partnership program with Lane Community College. Two of the students transferred to the U of O and continued to work in the lab – one of them, a non-traditional first generation student, went on to graduate school. A letter is included in the supplemental documents section from the director of the LCC internship program expressing interest in continuing to send students from underrepresented groups to our lab.

In addition to the 4 undergraduate students who will be paid for by this grant, we will apply each year for REU student funds. In each of the years of our last grant period we applied for and received REU supplemental funding. In addition, we typically have several undergraduate students who are not paid but working in the lab for research credit or experience. In total, 23 undergraduate students gained research experience during the course of our most recent grant, and we expect similar numbers for the proposed grant period. In addition to recruiting students through LCC, we will continue our program of having senior students make recruiting presentations in U of O classes. Our most recent round of these presentations recruited 6 new undergraduates who began by volunteering in the lab. Finally we will use the U of O student employment system in conjunction with a Cal-Tech SURF student rating system, that we have had experience with, that is designed to increase representation of under-represented groups without being unfair to others. In addition to at least 20 undergraduate students, we will train 1 graduate student.

Our students tell us that one of the reasons that working in our lab is popular on campus is that we have a reputation for close collaboration between senior personnel and undergrads. Although we will use senior students to train new undergraduates, each student will collaborate directly with senior personnel on their projects. Close mentorship improves training and results in more opportunities to make significant intellectual contributions to papers. For example, one of our first generation student undergraduates is currently writing a paper and will serve as lead author.

For each of the years of the previous grant, we participated in the summer Quark Net (http://quarknet.fnal.gov) continuing education program for high school science teachers. Teachers have said that the interdisciplinary nature of our research has helped them make connections for their students between physics and biology. We plan on continuing our Quark Net presentations and lectures for each year of the proposal period.

OUTREACH

Since this topic is so simple and so universal (e.g., "Every ant, every spider you see has these strange little Zn teeth that may someday help us build microscopic medical machines"), it makes a good candidate for exciting students and the general public about material science and biology, promoting interest and scientific education. About 20 million listeners heard radio interviews with the PI about our most recent NSF-sponsored research, IOS 0422234, and the results were featured in print publications with a readership of more than 50 million. Programs that interviewed us about behavioral correlates with wear (links here), included the BBC World Service, Voice of America, NPR's Science Friday (video pick of the week), CBC Quirks and Quarks, BBC 5 Live Drive, and, most importantly according to some of the co-authors, our results were featured in a limerick on NPR's "Wait, Wait Don't Tell Me". Summaries of this research in printed media included a page in the June 2011 National Geographic. Our research on mechanical properties of heavy-element materials was featured on more than 60 websites, and we were interviewed on the topic for the AAAS radio show. The biomaterial research was also featured in an NSF Highlight. We attribute our outreach successes to our ongoing relationship with the U of O communications office and their excellent contacts in the media. We will continue to provide material for press releases to the communications office for each of the major results from the proposed research.