doi:10.1017/S1431927614008393
Microsc. Microanal. 20 (Suppl 3), 2014

STEM Characterization of Nano-Crystallites in the Nacre Biomineralization of Mollusk Shells *(Pinna nobilis)*

Robert Hovden[1], Stephan E. Wolf[2,3], Megan E. Holtz[1], David A. Muller[1], Lara A. Estroff[2]
[1] School of Applied & Engineering Physics and Kavli Institute at Cornell University, Ithaca NY
[2] Department of Materials Science and Engineering, Cornell University, Ithaca NY
[3] Department of Materials Science and Engineering, Institute of Glass and Ceramics, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany

The intricate biomineralization processes in mollusks lead to hierarchical structures with micro-, nano-, to atomic scale features. Comprised of calcium carbonate ($CaCO_3$) polymorphs—primarily, aragonite and calcite—mollusk shells have a variety of structures [1,2,3]. Most familiar of which are the illustrious mother-of-pearl (nacre) layers. In simple prismatic species (e.g. in the order of Pterioida), aragonite nacre forms atop an outer shell assembled from an array of calcite prisms [4,5]. However, formation and transition from prismatic calcite to nacreous aragonite is a poorly understood biomineralization process.

Here we observe nanoscale details of the formation of nacre following the termination of prismatic growth in an endemic Mediterranean Noble Pen Shell, *Pinna nobilis*—a representative prism-to-nacre mollusk species. We observe nano-crystallites within a thick intra-organic layer that appear to initiate nacreous growth (Fig 1c). These crystallites aggregate with increasing density as the growth progresses until finally forming continuous aragonite nacre layers (Fig 1 c,d). This process can occur over several microns; however, the initial crystallites are roughly 50 nm and thus are not directly observable by optical or x-ray techniques. Thus, observing assembly mechanisms in early nacre formation is easily missed without the aid of electron microscopy.

We employed high-resolution scanning transmission electron microscopy (STEM) with annular dark field (ADF), bright field (BF), and electron energy loss spectroscopy (EELS), to observe the onset of early nacre biomineralization in a mollusk with sub-nanometer resolution. While a cross sectional view shows layers in the growth process (Fig 1), the structure and packing density of the nanocrystallite aggregation (Fig 1d *dashed line)* is more clearly seen from the planar sectioning (Fig 2a,b). Polycrystallinity within particles is most visible in bright field STEM images (Fig 2d)—where contrast within a particle is sensitive to crystallographic orientation and structural defects. Convergent beam and selected area electron diffraction (CBED & SAED) confirmed polycrystalline structure throughout the crystallites and found them most consistent with the aragonite structure (Fig. 2c).

In this work, electron microscopy greatly elucidated the structural origin of nacre in mollusks. Polishing methods were invaluable for preparation of electron-transparent mollusk specimens—both in cross section and planar geometries. This dual geometry approach allowed deduction of the three-dimensional growth processes at the transition from prismatic to nacreous growth. The high resolution STEM allowed observation of a previously unknown aggregation layer.

[1] J.D. Taylor, M Layman, Paleontology **15,** 73 (1972)
[2] J. G. Carter, Skeletal Growth of Aquatic Organisms. Plenum, New York NY, (1980)
[3] H. Li, H. L. Xin, D. A. Muller, L. A. Estroff, Science, **326,** 1244 (2009)
[4] W. B. Carpenter, Rep. Brit. Ass. Adv. Sci. **14,** 1 (1844)
[5] I C. Olson, et al., J. Struct. Biol., **183,** 180 (2013)
[6] Funded by CCMR NSF MRSEC DMR-1120296 and DMR-0845212

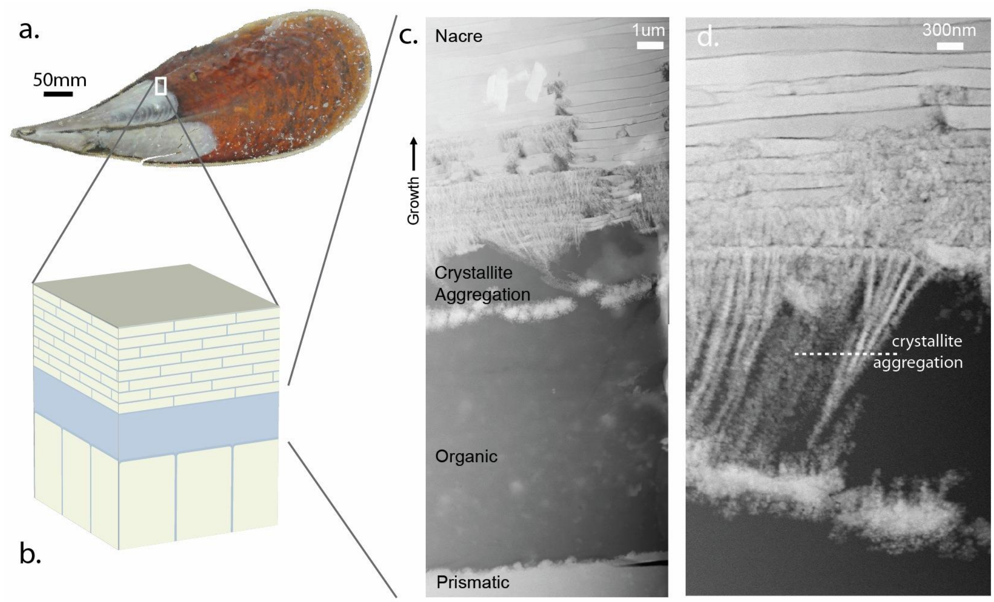

Figure 1 Cross-sectional view of the transition from prismatic to nacreous growth. a) *Pinna nobilis* mollusk shell from which the specimen was prepared. Cartoon (b) illustrating the geometry of the nacre-prism interface. c) ADF-STEM image showing the nanoscale growth of nacre via crystallites within an organic matrix. d) Aggregation of crystallites leads to the onset of nacre layers. Crystallite aggregation and packing plane highlighted with dashed line.

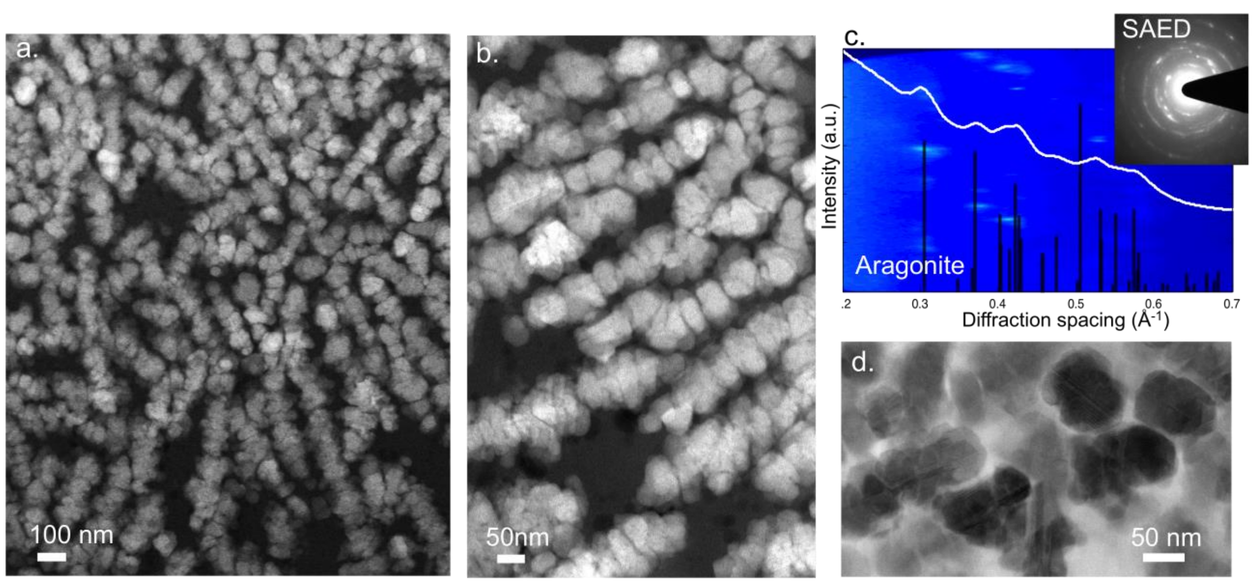

Figure 2 Planar view of the nanocrystallite packing in crystallite aggregation region. (a). ADF (b,c) and BF (d) of the nanocrystallites show polycrystallinity among the particles and within. SAED shows the crystallites are consistent with the aragonite $CaCO_3$ polymorph—i.e. the crystal structure of nacre. c) plot of radially integrated SAED intensity with known aragonite peaks marked along the x-axis.

https://doi.org/10.1017/S1431927614008393 Published online by Cambridge University Press