Understanding the Microarchitecture of Mother-of-Pearl

S.N. Coppersmith
Department of Physics
University of Wisconsin, Madison

Faculty collaborator:
Prof. Pupa Gilbert

Lead grad student:
Rebecca Metzler

but first ....

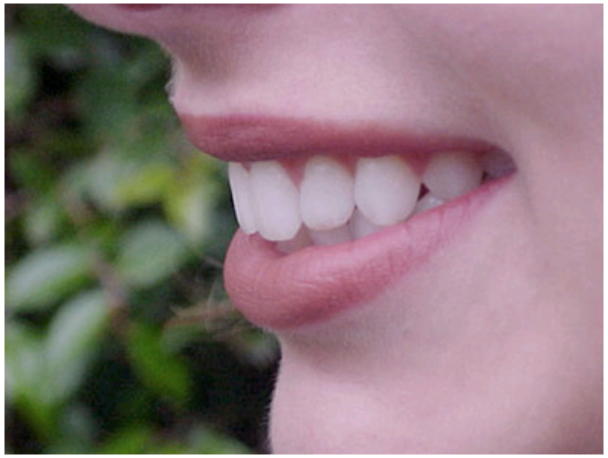
A little bit about me

Employment:	Professor of Physics, University of Wisconsin–Madison	2001-present
	Professor of Physics, University of Chicago	1995-2001
	Distinguished Member of Technical Staff, AT&T Bell Laboratories	1990-1995
	Member of Technical Staff, AT&T Bell Laboratories	1987-1990
	Visiting Lecturer, Princeton University	1986-1987
	Postdoctoral Member of Technical Staff, AT&T Bell Laboratories	1985-1986
	Research Associate, Brookhaven National Laboratories	1983-1985
Education:	Cornell University, Ithaca, NY	1979-1983
	M.S., Physics, 1981; Ph.D., Physics, 1983	
	Cambridge University, Cambridge, U.K.	1978-1979
	Applied Mathematics Tripos, Part III	
	Massachusetts Institute of Technology, Cambridge, MA	1974-1978
	B.S., Physics, 1978	

Biomineralization

The process through which living organisms create structures out of minerals and organic molecules

- Bones
- Teeth
- Shells



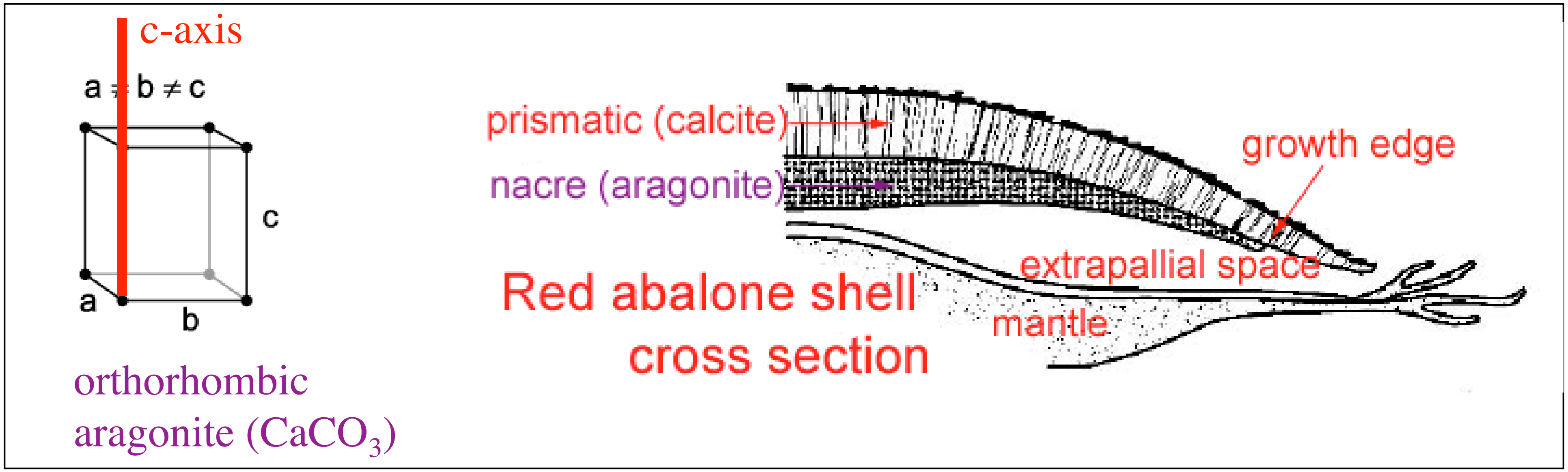
This work: abalone nacre

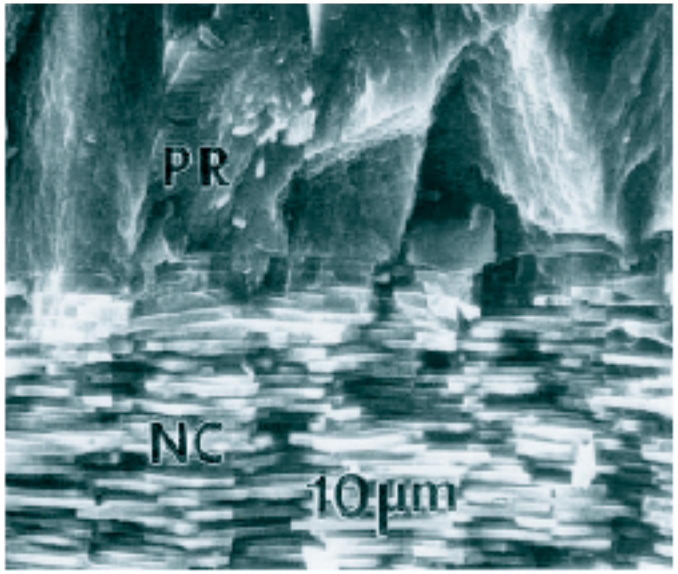
- Nacre: the shiny inside of the abalone shell (shiny because of its layered structure: coherent interference between light reflection off different layers)
- Nacre is 3000 times more resistant to fracture than aragonite, even though it is 95% aragonite

Why this topic?

It shows how physics techniques and concepts can yield new insight into many other scientific fields (in this case biomineralization, which is usually studied by materials scientists and geologists).

Red Abalone (*Haliotis rufescens*)



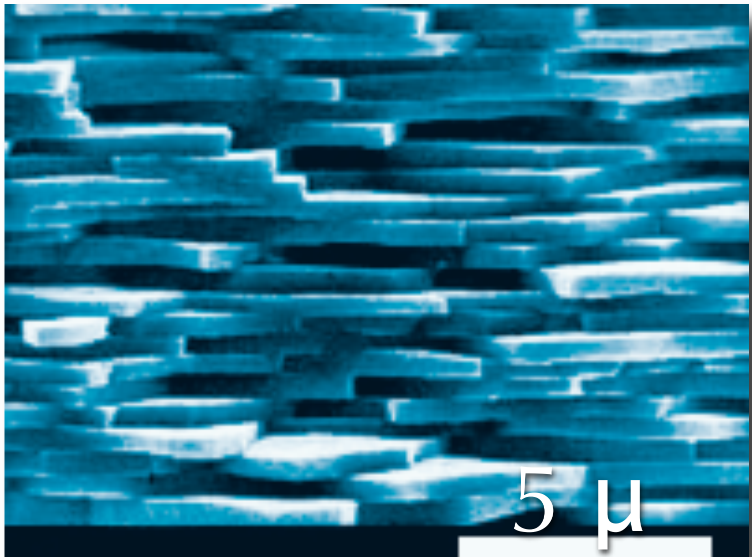


Scanning electron micrograph (SEM) of fractured shell, showing both prismatic and nacrous regions

E. DiMasi, M. Sarikaya, J. Mat. Res. 19, 1471 (2004)

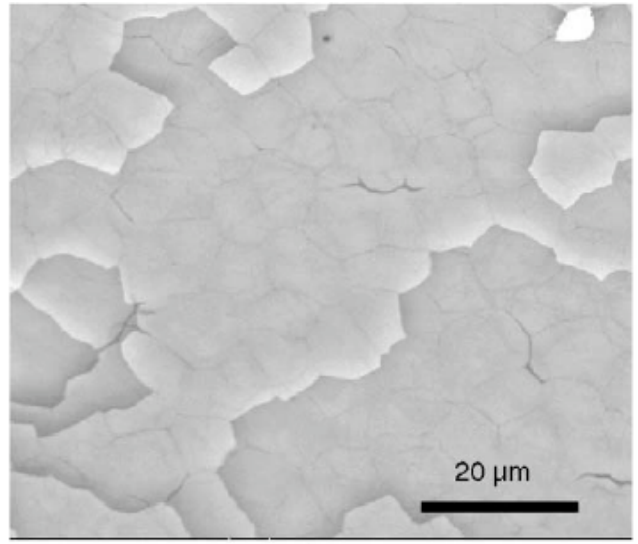
abalone nacre

Side view



P. Gilbert, (unpublished)

Top view

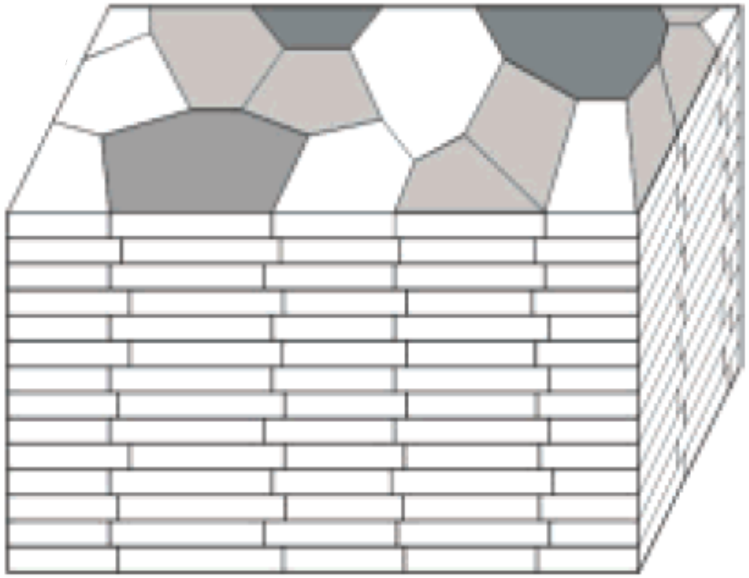


A. Lin and M.A. Meyers, Mat. Sci. Eng. A 390, 27-41 (2005)

Layers of aragonite tablets (~5 μm transverse dimension, 0.4 μm thick) in an organic matrix ~30 nm thick

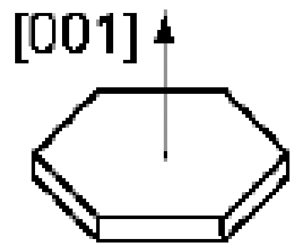
P. Gilbert, et al., in Reviews in Mineralogy & Geochemistry 59, 157 (2005)

Nacre structure



Brick (mineral) and
mortar (organic) structure

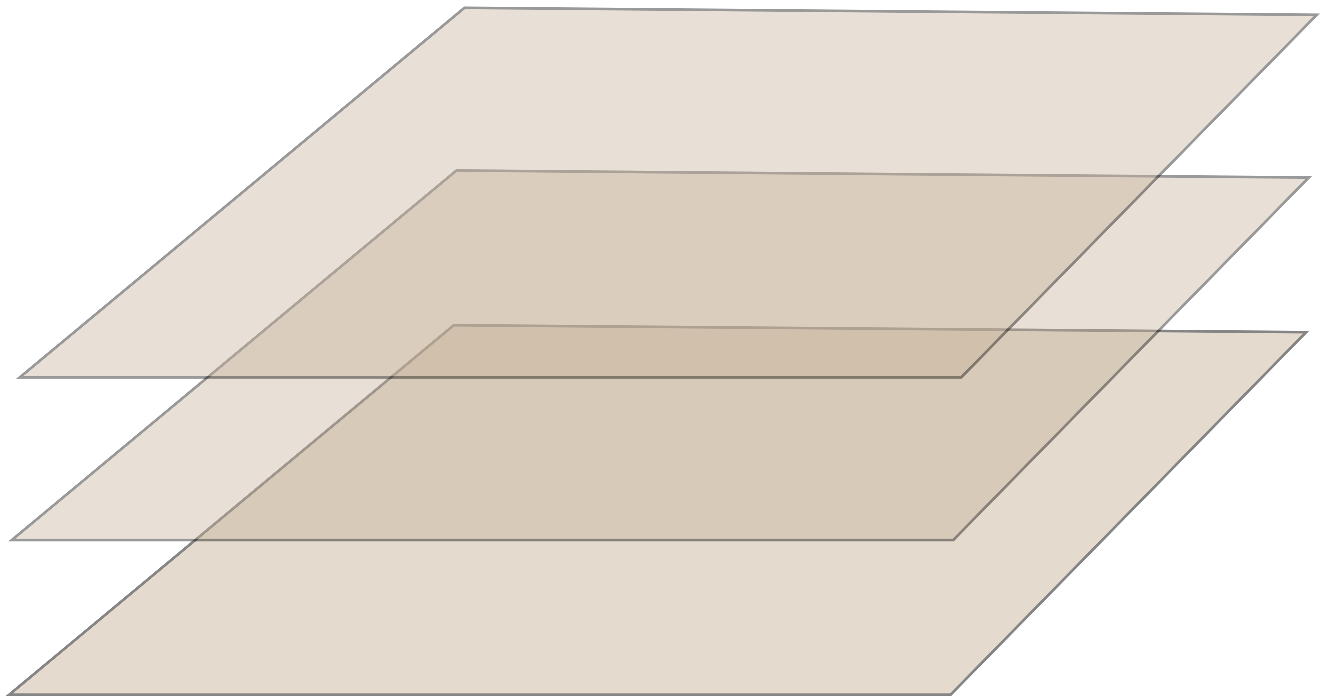
Nacre is ~95% aragonite
and ~5% organic



X-ray data show that the c-axes of aragonite tablets are aligned to within ~5°, and ab-planes of tablets are disordered.

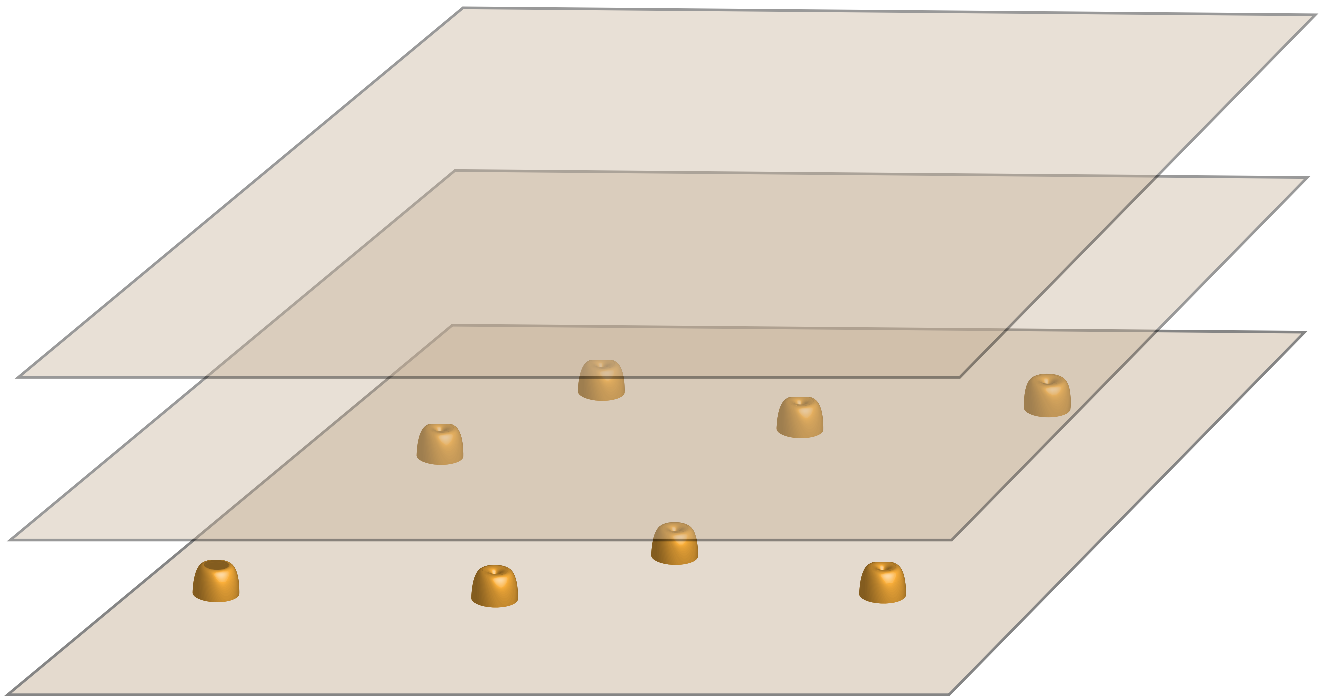
Nacre growth

First, organic matrix layers are created.



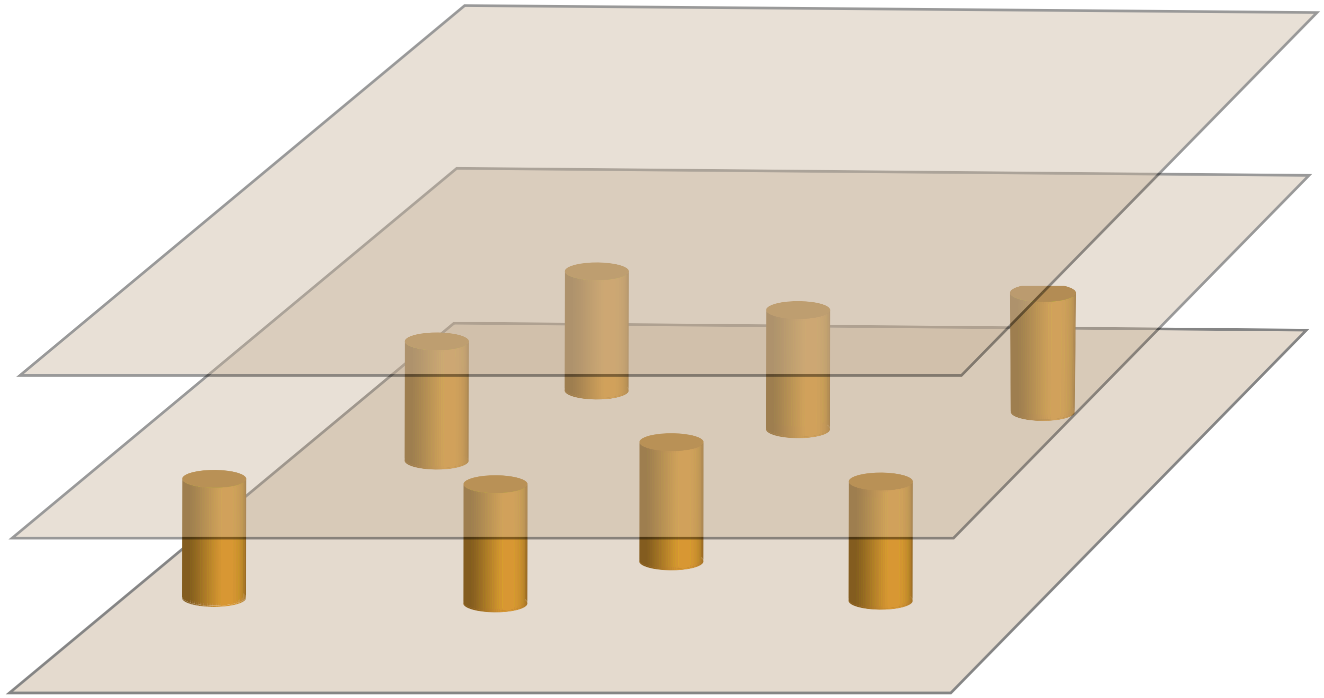
Nacre growth

Then, first layer of aragonite tablets nucleates.



Nacre growth

Then, the aragonite tablets grow, restricted by organic layers.



Nacre growth

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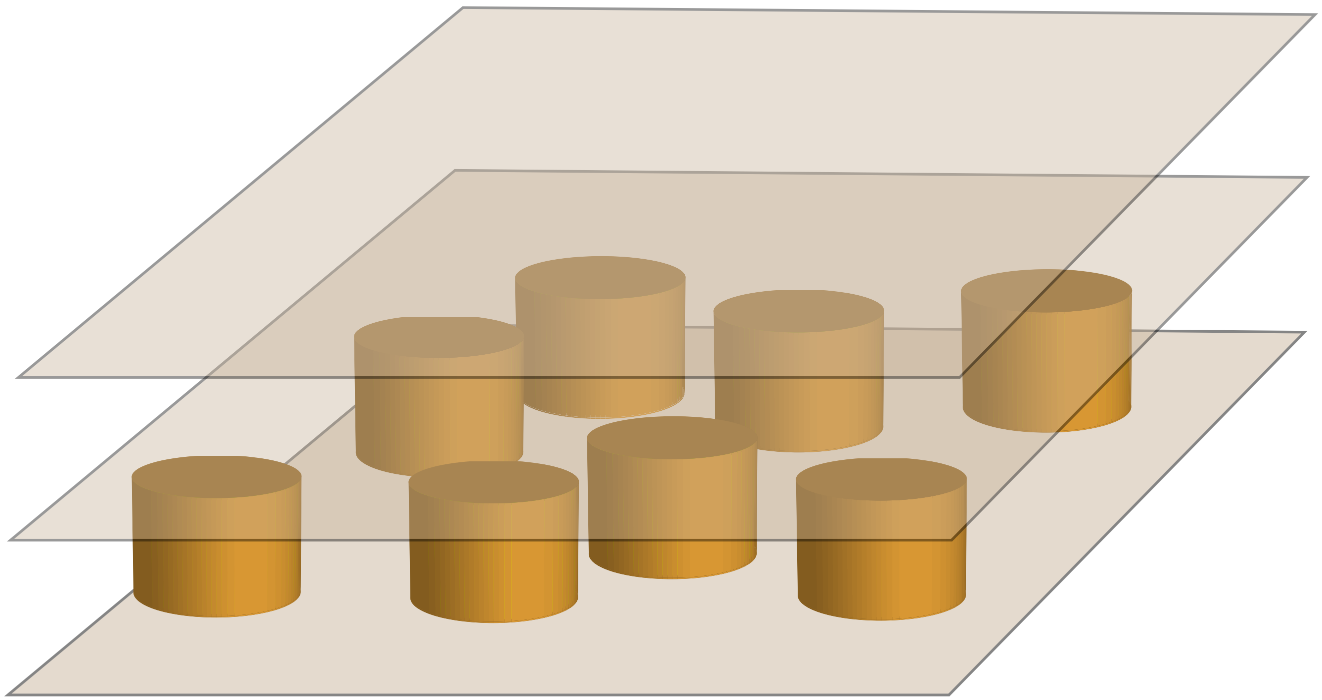

Nacre growth

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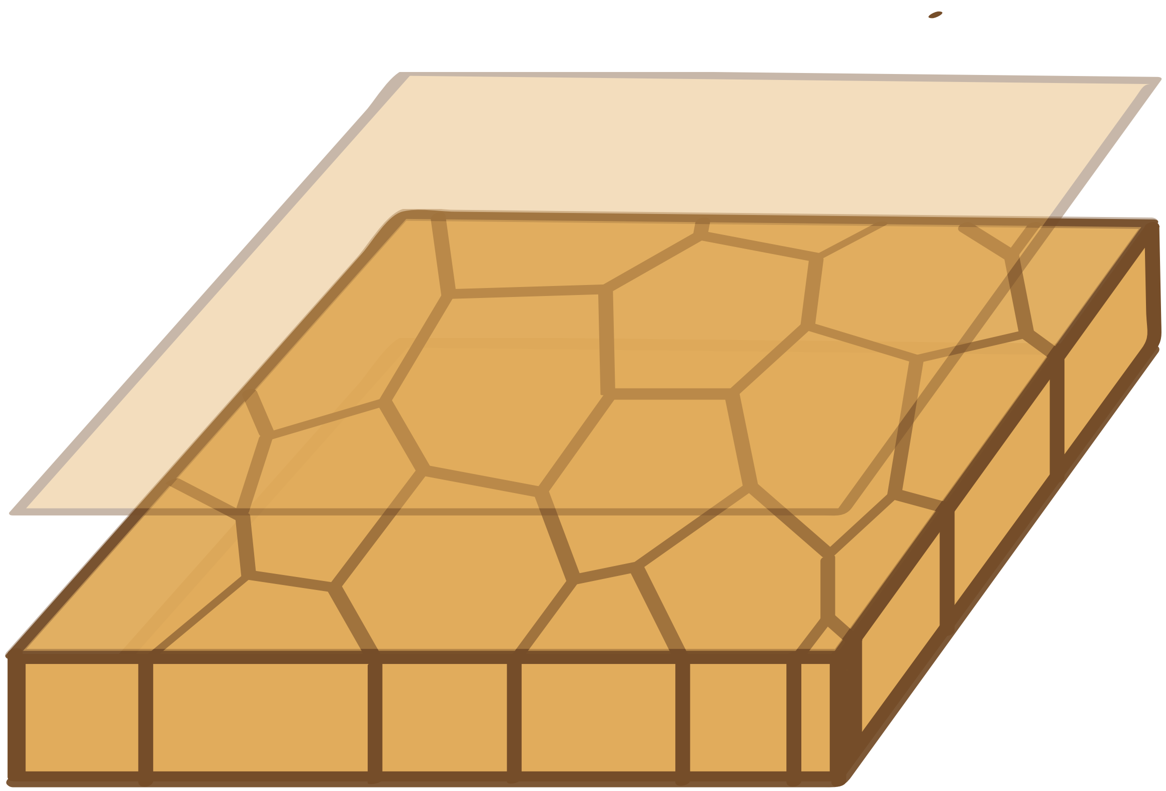
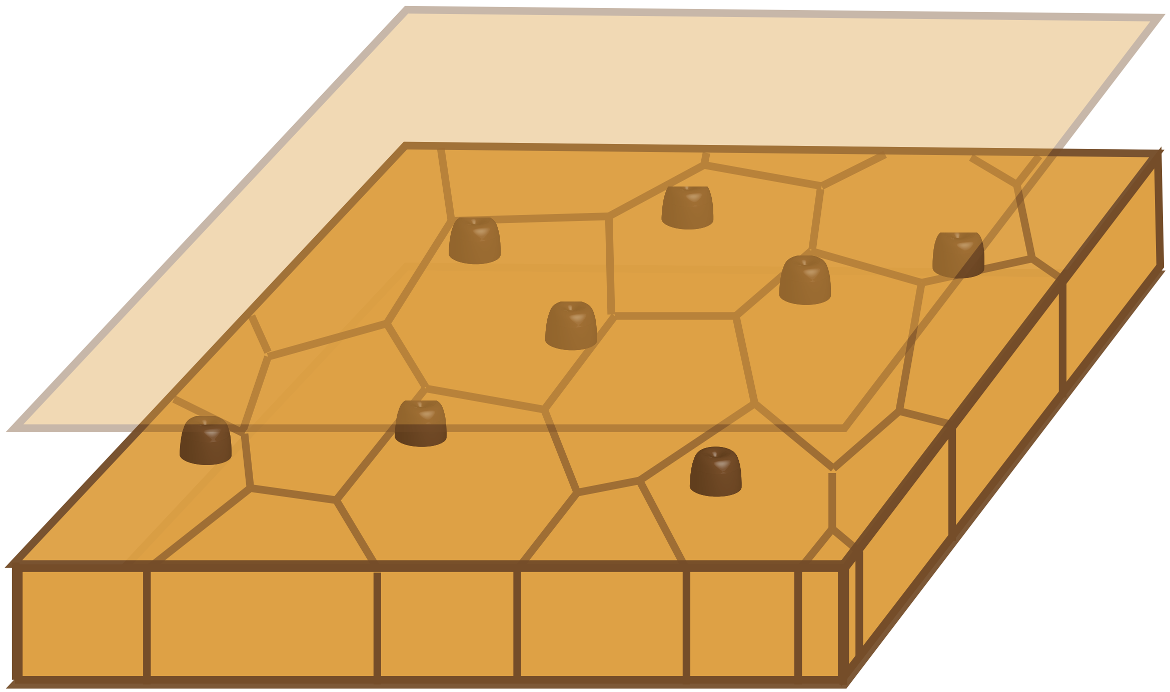


Nacre growth

After tablets in first layer grow to confluence, nucleation starts in second layer.



Question addressed here:

What is the mechanism that aligns aragonite tablets in nacre so the crystal c-axes are perpendicular to the layers?

- Previous belief: crystal tablets align because of chemical templation by organic layers
- This work: alignment is result of dynamical process of directed assembly

It has been believed that aragonite tablets in nacre are oriented stereochemically by organic matrix layers, but evidence for this mechanism is limited.

Table 1. Percentage of (001)-oriented calcite crystals nucleated on polystyrene films with different treatments

Polystyrene film	Adsorbed polymer	(001)-oriented calcite crystals, %	
		Without polymer	With polymer
Sulfonated 0.5 min	Poly(aspartic acid)	3.0 ± 0.5	2.5 ± 1.0
Sulfonated 1 hr	Poly(aspartic acid)	7.5 ± 3.0	8.5 ± 4.0
Sulfonated 24 hr	Poly(aspartic acid)	7.0 ± 4.0	25 ± 7.0
Culture dish	Pure protein	7.4 ± 1.5	14.6 ± 3.9
Sulfonated 0.5 min	Protein assemblage	3.0 ± 0.5	17.5 ± 6.5
Sulfonated 0.5 min	Protein assemblage (blocked carboxylate)	3.0 ± 0.5	5.7 ± 0.6

L. Addadi et al., PNAS 84, 2732 (1987)

see also Heinemann, Treccani, Fritz, Biochem. Biophys. Res. Commun. 344, 45-49 (2006)

Experimental technique: X-ray Absorption Near Edge Structure (XANES) spectroscopy

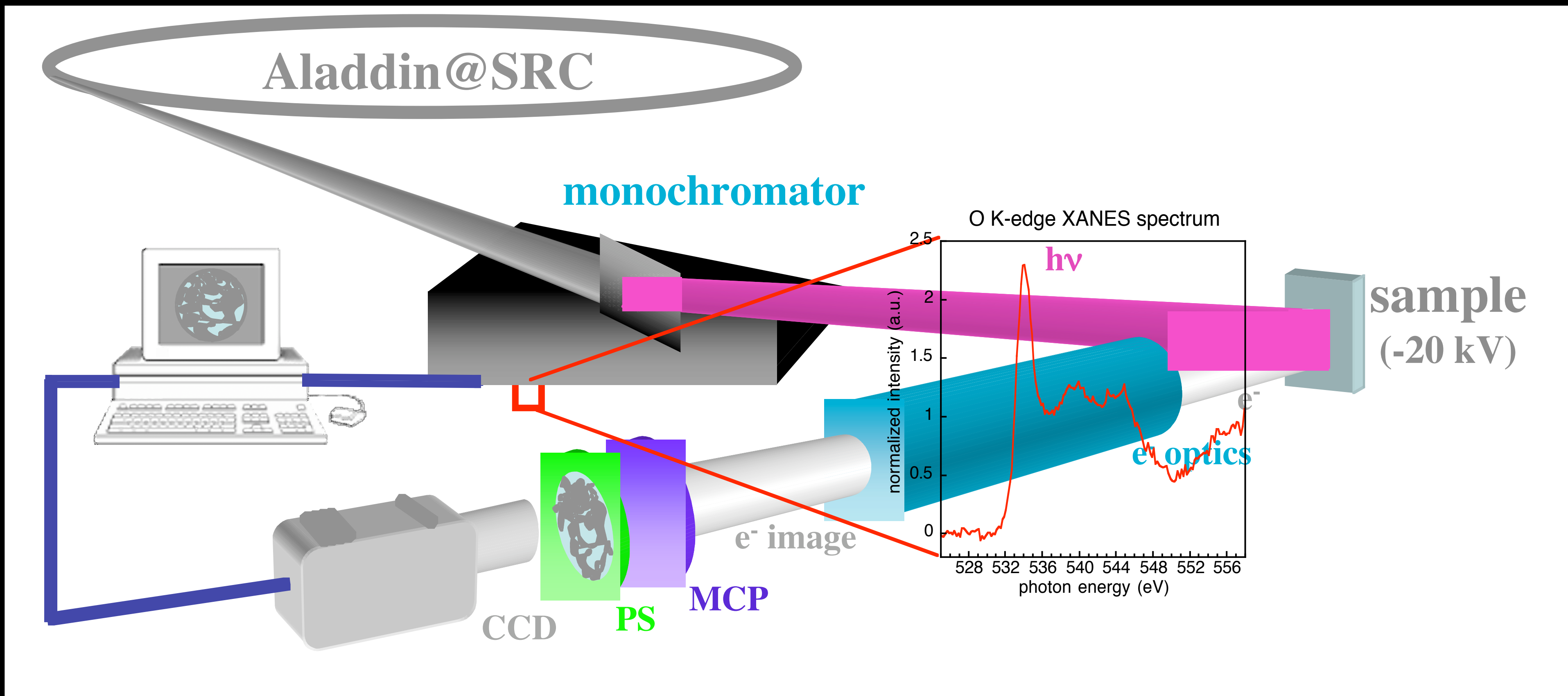
XANES is spatially-resolved photoemission (one measures electrons that are emitted when x-rays are directed onto sample)

XANES spectra yield information about chemical bonds in a material, and can be used to:

- distinguish bond types (π versus σ)
- detect oxidation states
- examine structural differences based on bonding environment

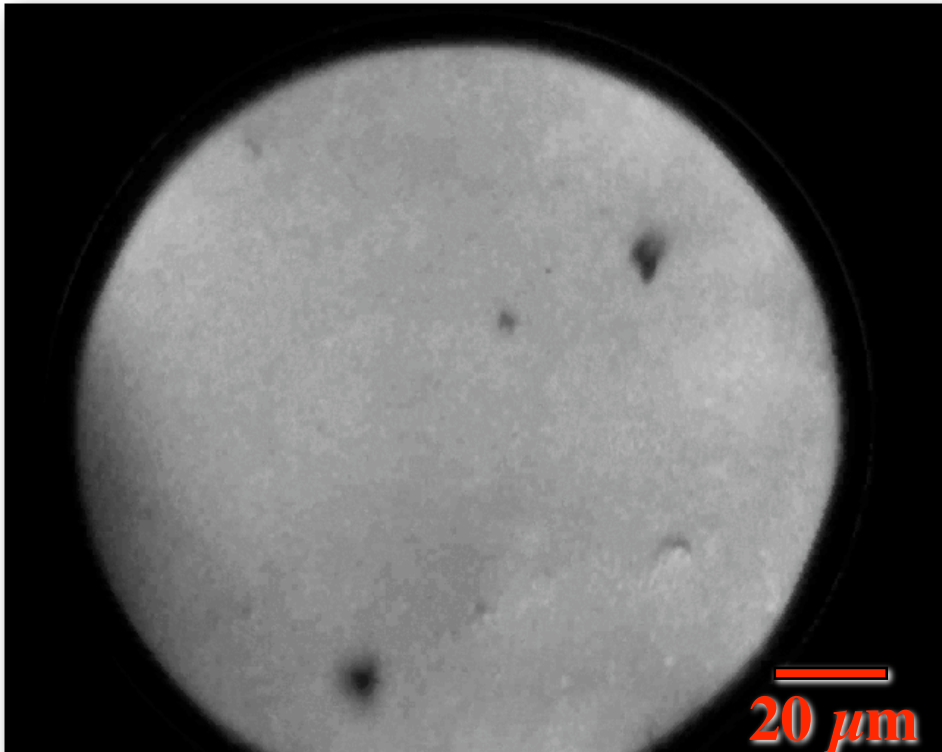
SPHINX

(Spectromicroscope for PHotoelectron Imaging of Nanostructures with X-rays)

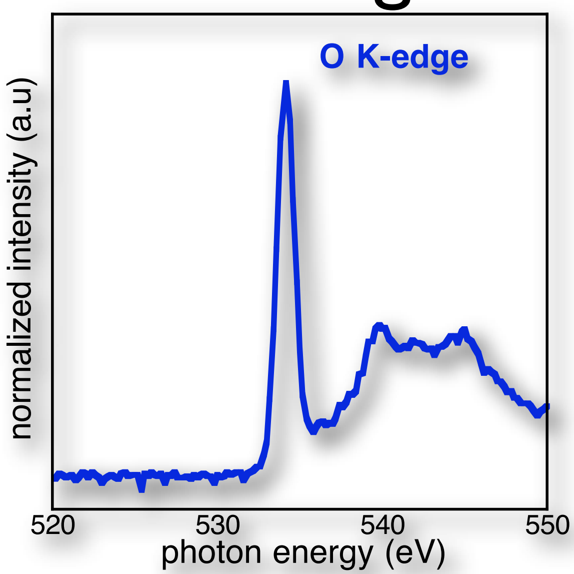


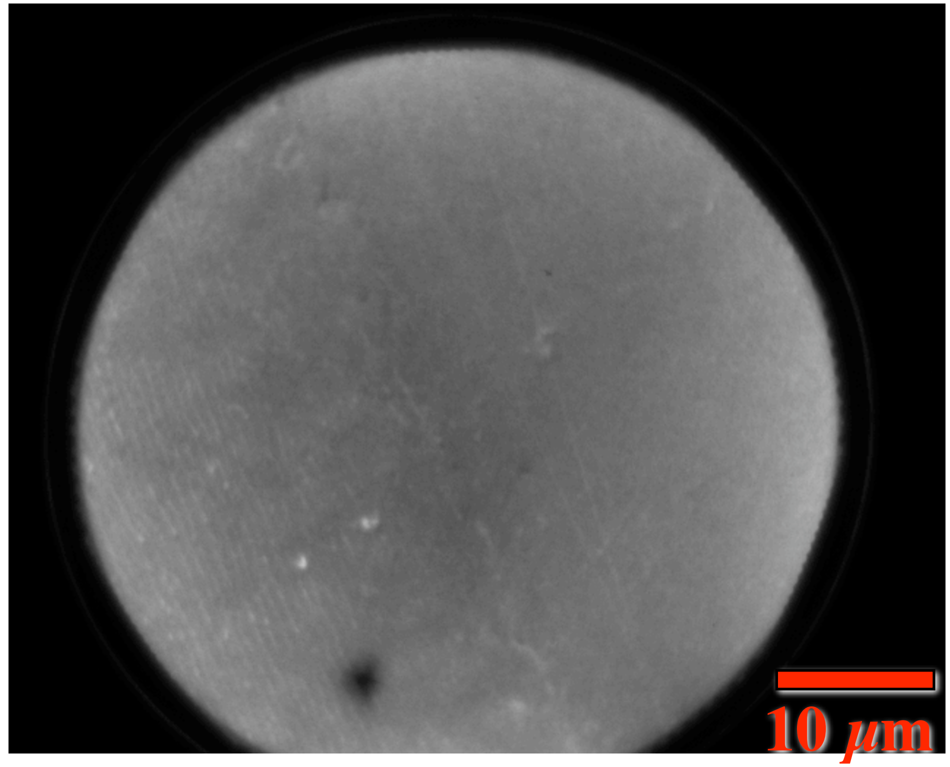
X-ray PhotoElectron Emission spectroMicroscopy (X-PEEM) with XANES

Movies of XANES spectra of abalone nacre

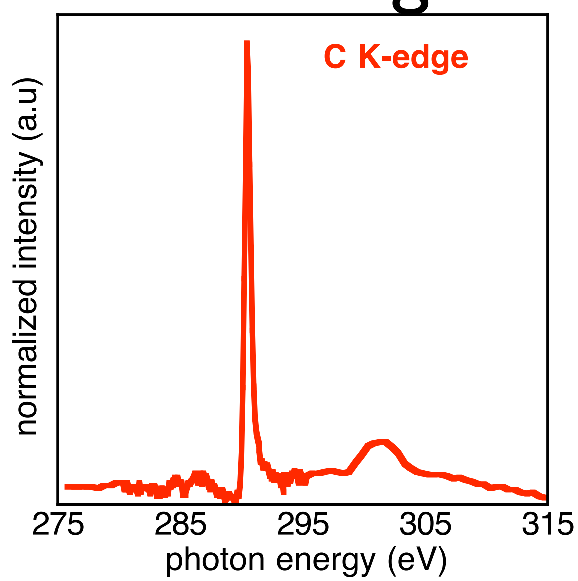


O K-edge



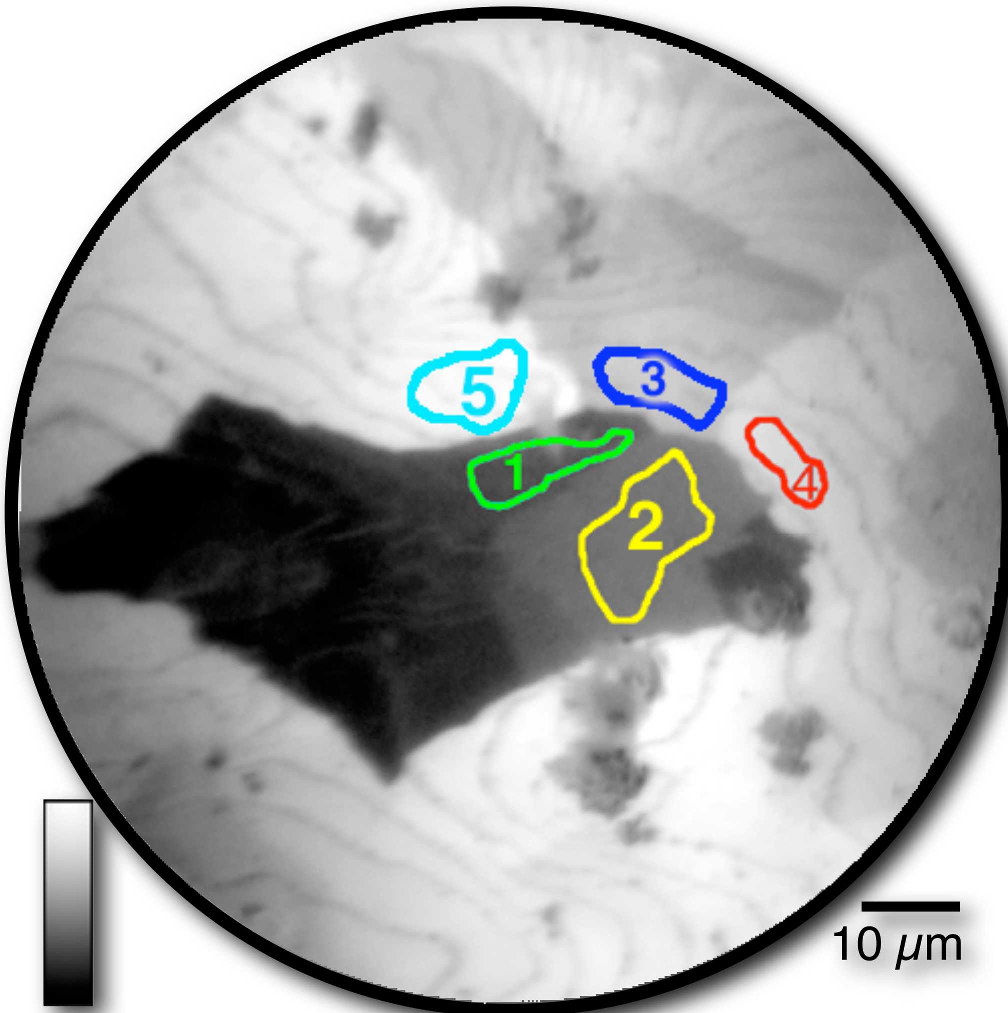


C K-edge

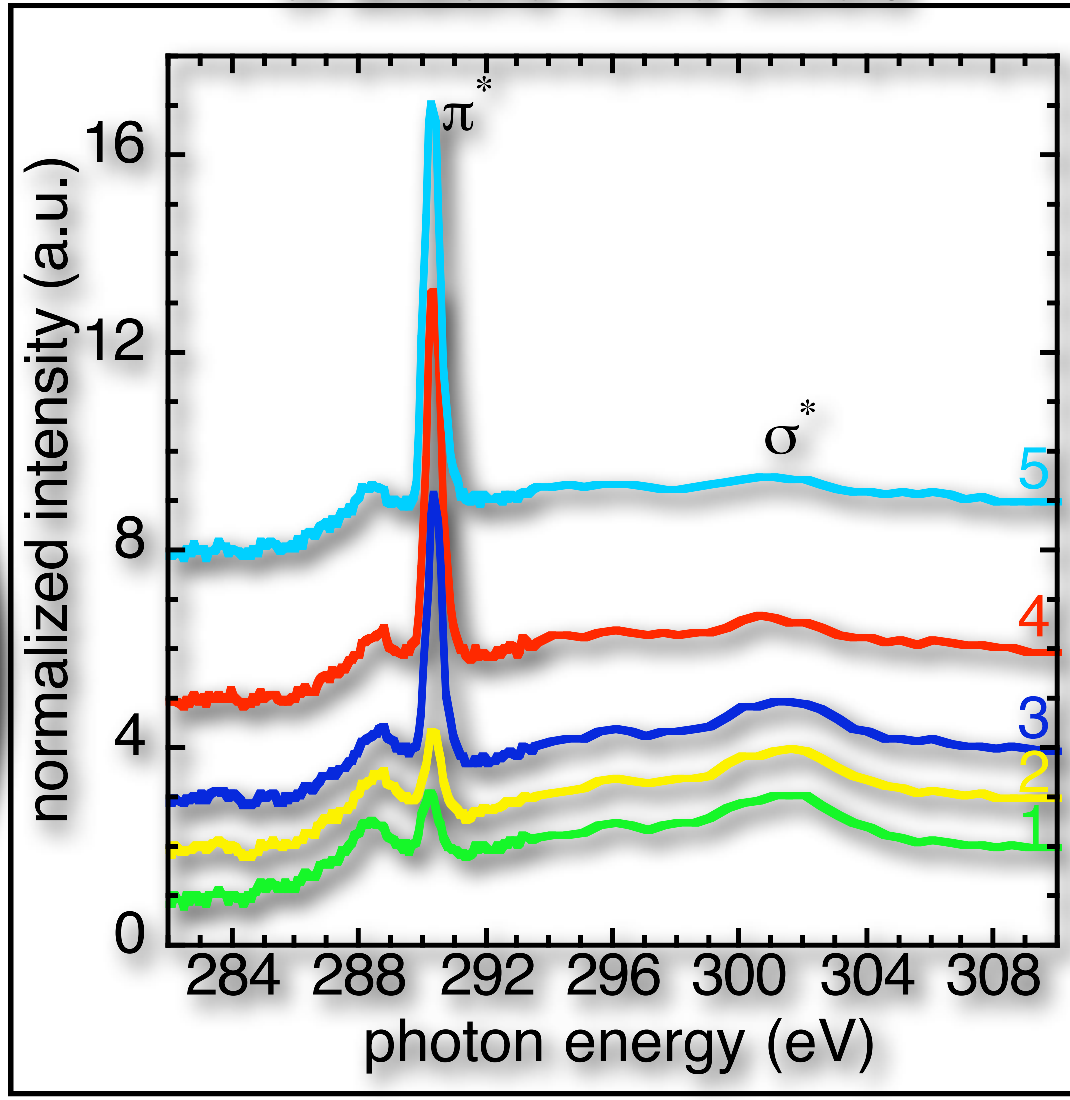


Abalone nacre

C map (π*/σ*)



Carbon K-edge XANES spectra of abalone nacre tablets



contrast is due to different single-tablet crystal orientations

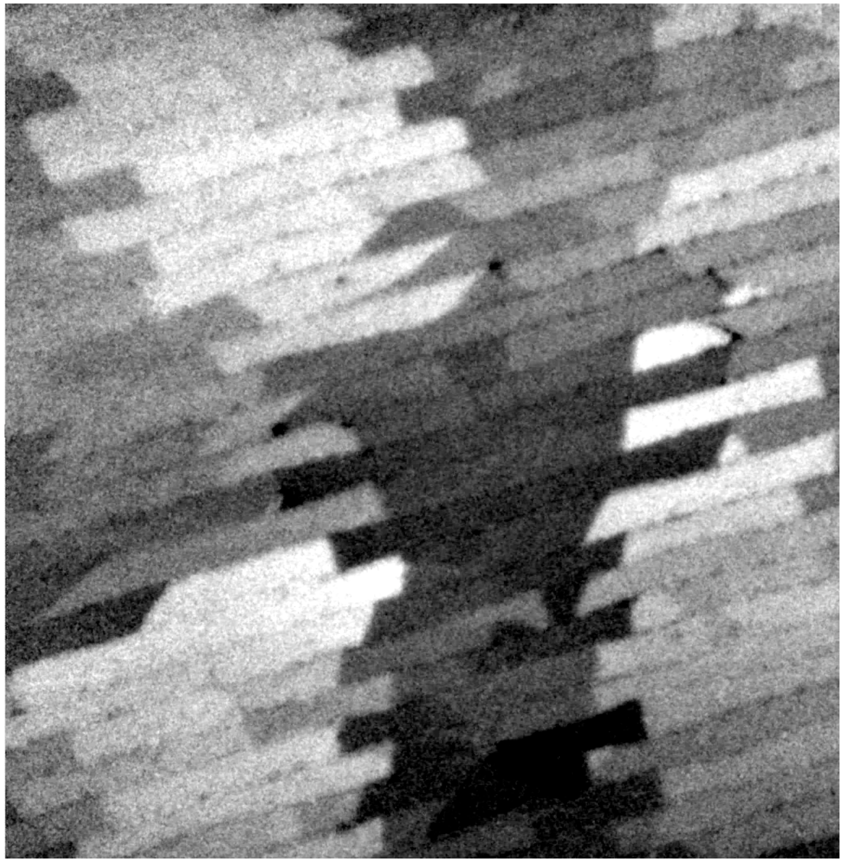
RA Metzler et al., *PRL* **98,** 268102 (2007).

XANES spectra are sensitive to chemical bond directionality, so yield information about aragonite tablet orientations

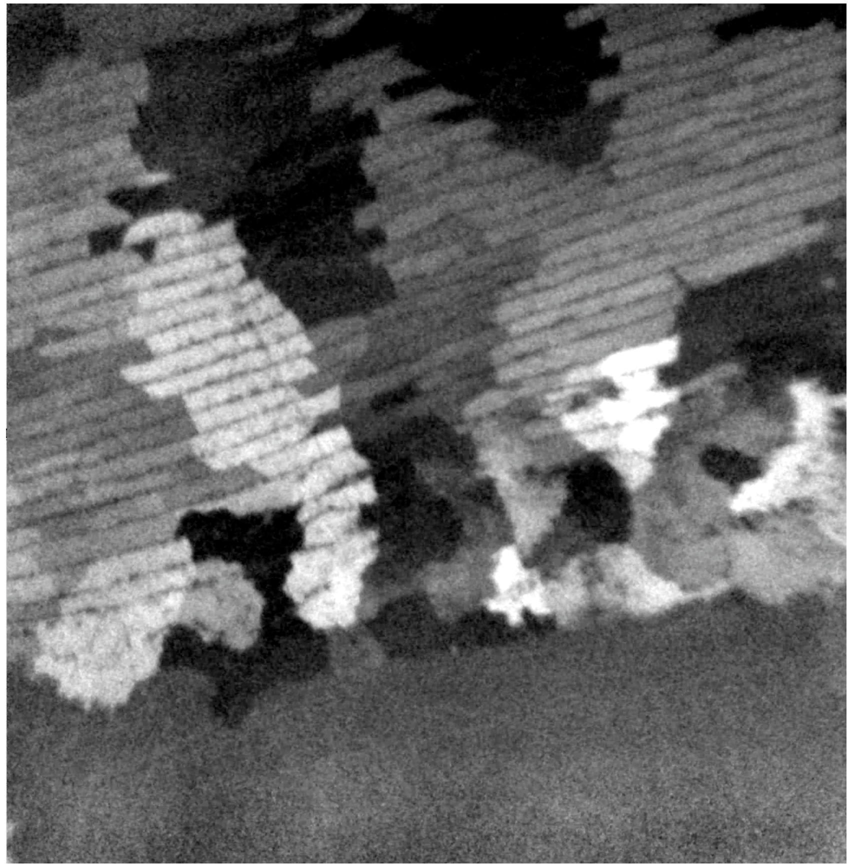
Intensity $\propto \cos^2\Theta$, where Θ is the angle between the aragonite crystal c-axis and the radiation polarization (Stöhr 1992)

- ▷ Contrast ⇒c-axes of different aragonite tablets not aligned
- ▷ C-axes of tablets align moving away from prismatic boundary

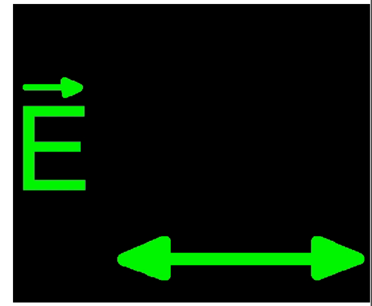
Experimental demonstration that contrast arises from differences in aragonite crystal orientation



Carbon π*/σ* maps
Field of view = 15.6 μm

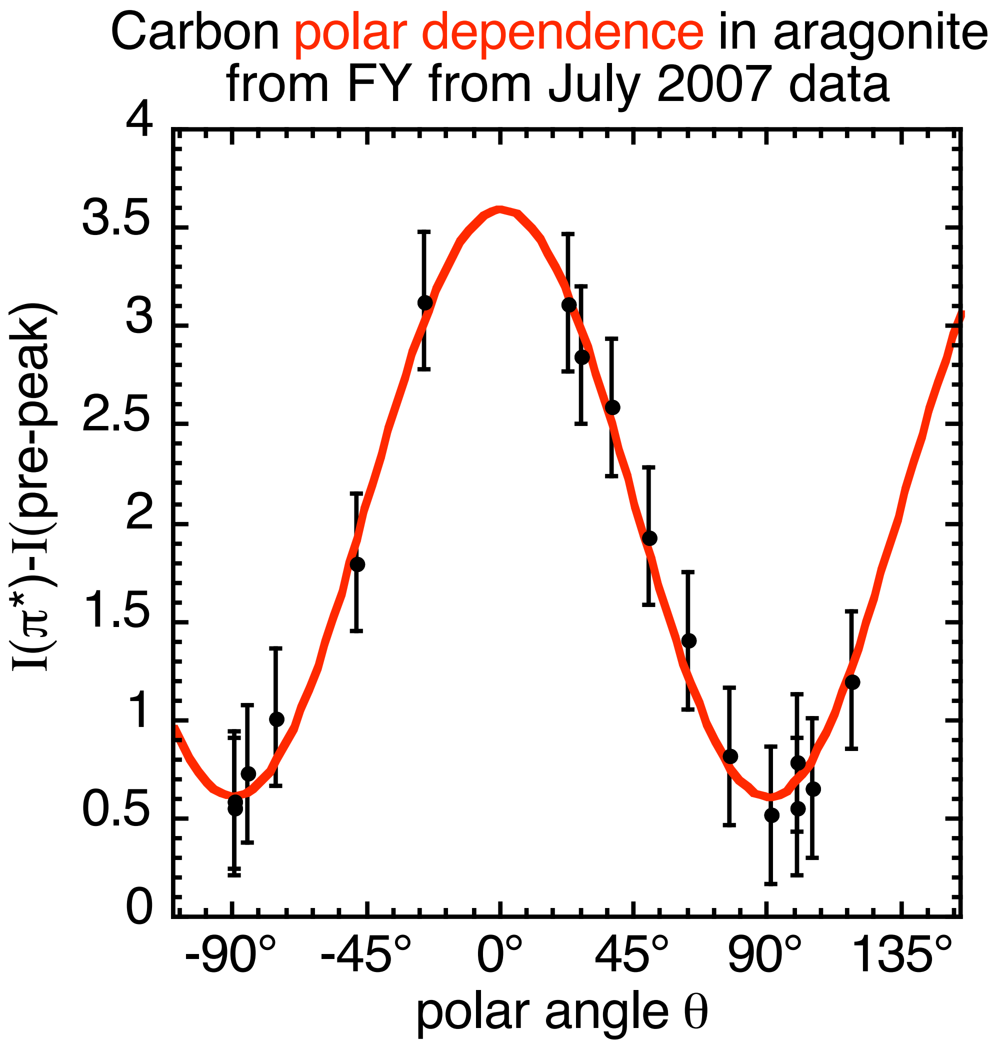


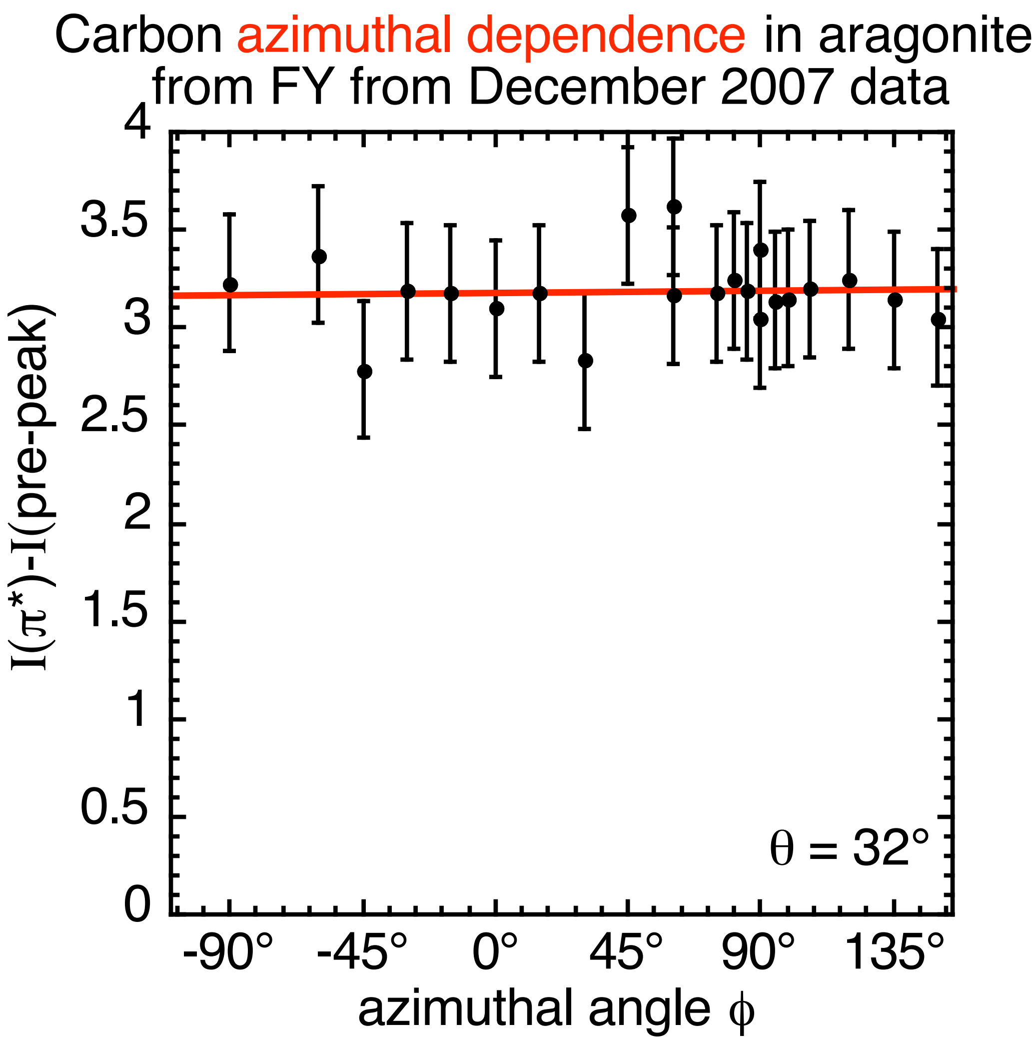
Carbon π*/σ* maps
Field of view = 23 μm



R. Metzler et al., unpublished (data taken at Advanced Light Source PEEM-3)

Dichroism in aragonite single crystals arises from polar angle variations and not azimuthal variations



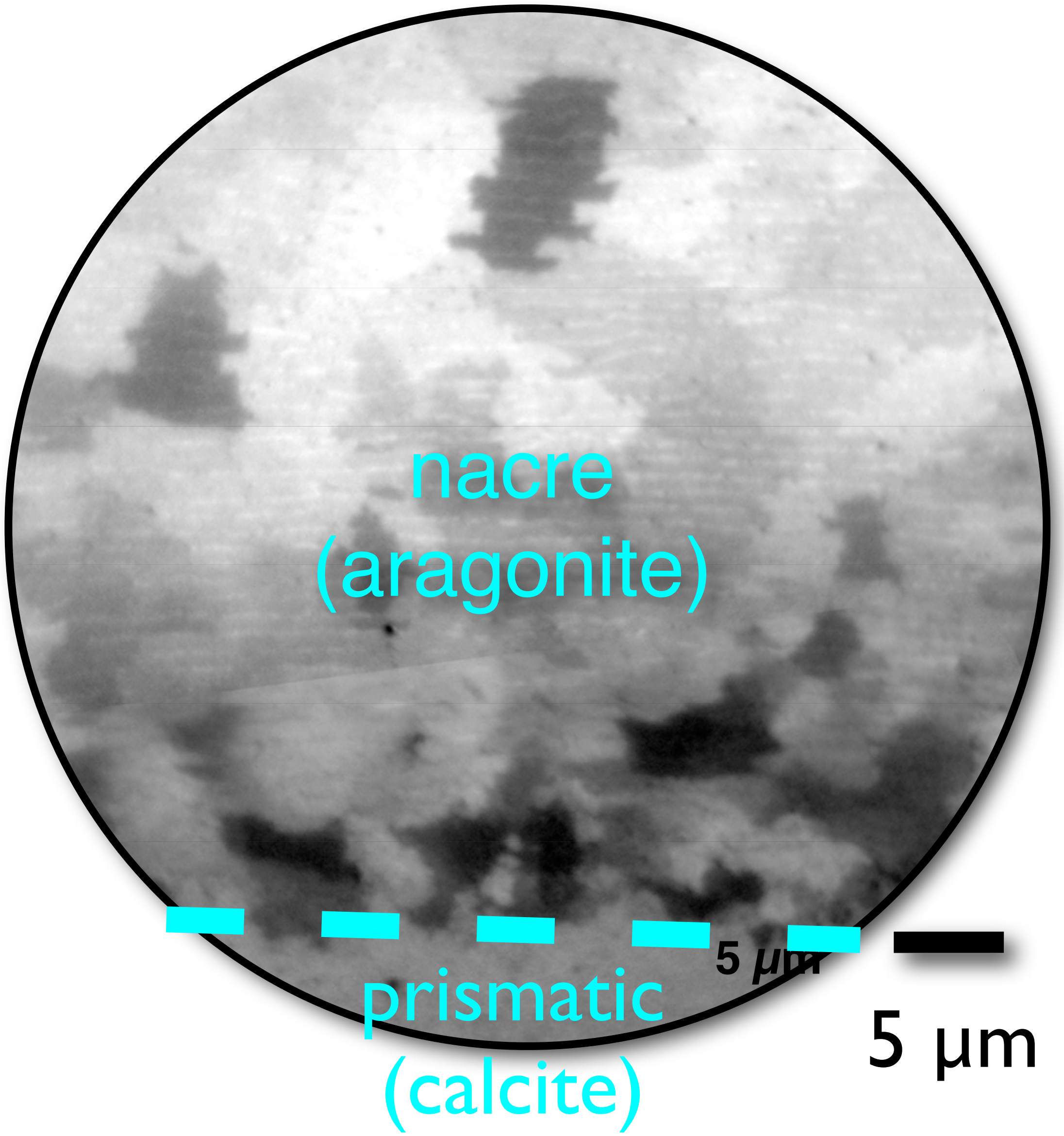


Sample: single crystal geologic aragonite

Nacre: Role of theory

- Theory clarifies contrast mechanism (contrast in XANES images arises from different c-axis orientations, not from disorder in ab-plane)
- Analyzing pattern of tablet sizes and orientations yields insight into how nacre grows: c-axis order arises from dynamical mechanism

X-ray linear dichroism reveals nacre structure: contrast is greatest near prismatic boundary



nacre growth direction

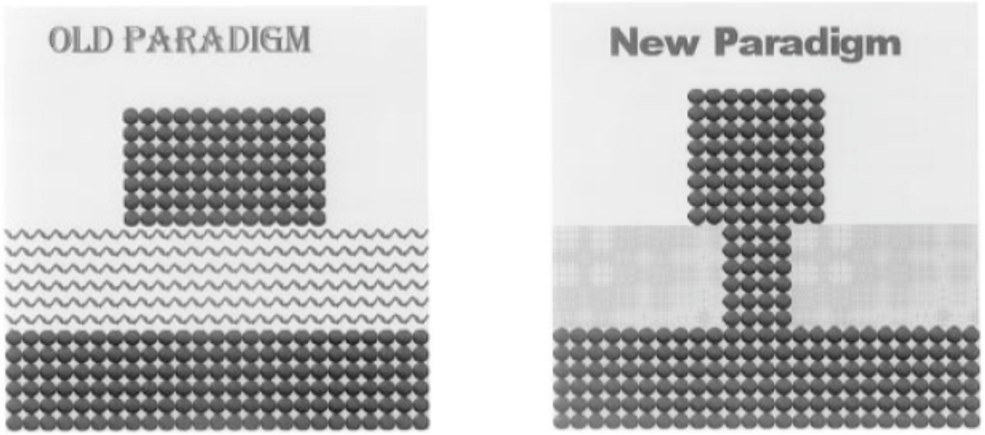
➡ c-axes of aragonite tablets become better aligned as nacre grows

R.A. Metzler, D. Zhou, M. Abrecht, S.N. Coppersmith, P. Gilbert, arXiv:0710.4573;
P.U.P.A. Gilbert et al., JACS, accepted for publication.

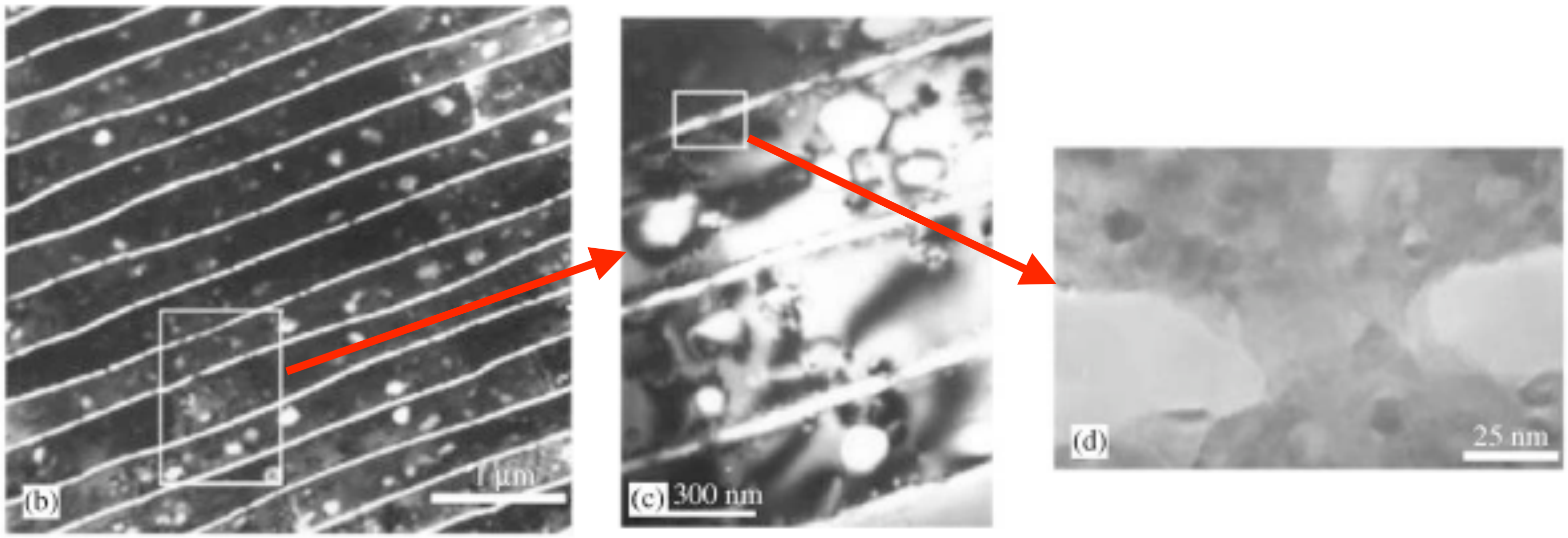
Dynamical model for development of orientational order in nacre

Model is based on the observation that mineral bridges between layers maintain crystal tablet orientation.

Mineral bridges between layers maintain aragonite crystal orientations



T.E. Schäffer, et al., Chem. Mater. **9**, 1731 (1997)



TEM micrographs

F. Song et al., Biomaterials **24**, 3623 (2003).

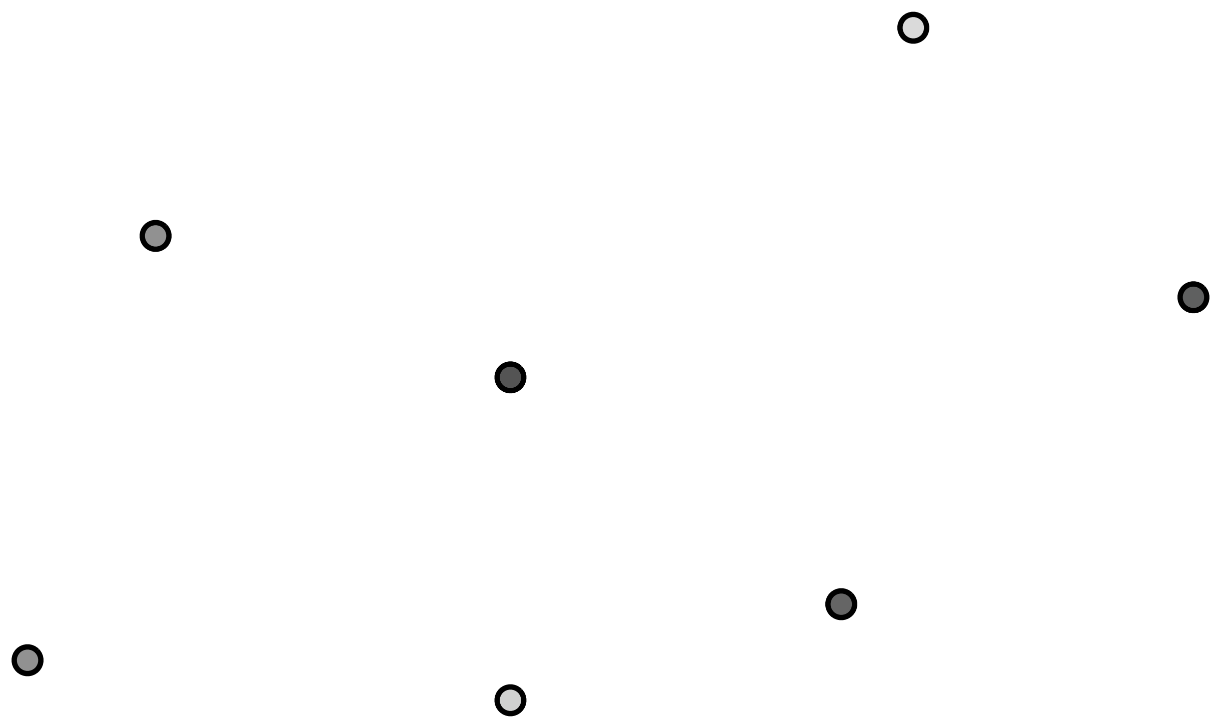
Mineral bridges can maintain crystal orientation.

Dynamical model of aragonite tablet alignment in nacre

Assumptions:

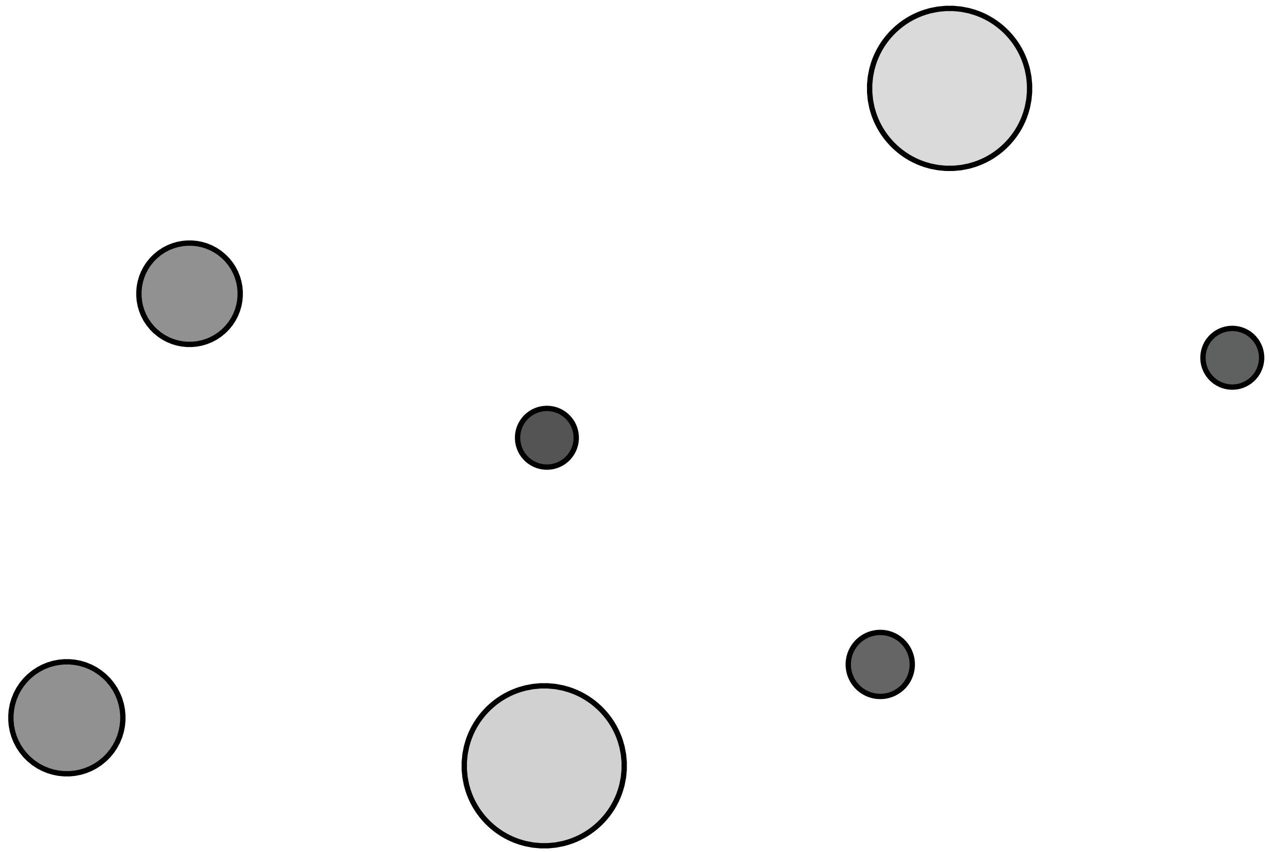
- Tablets with c-axes along (001) grow faster than tablets with other orientations; growth velocities in first layer vary between $(1-\delta)$ and $(1+\delta)$
- Growth proceeds layer-by-layer
- Nucleation site locations are random and uncorrelated
- Horizontal growth of each tablet in each layer is isotropic
- Each tablet takes on orientation of tablet below nucleation site with probability $(1-\epsilon)$

Cartoon of model

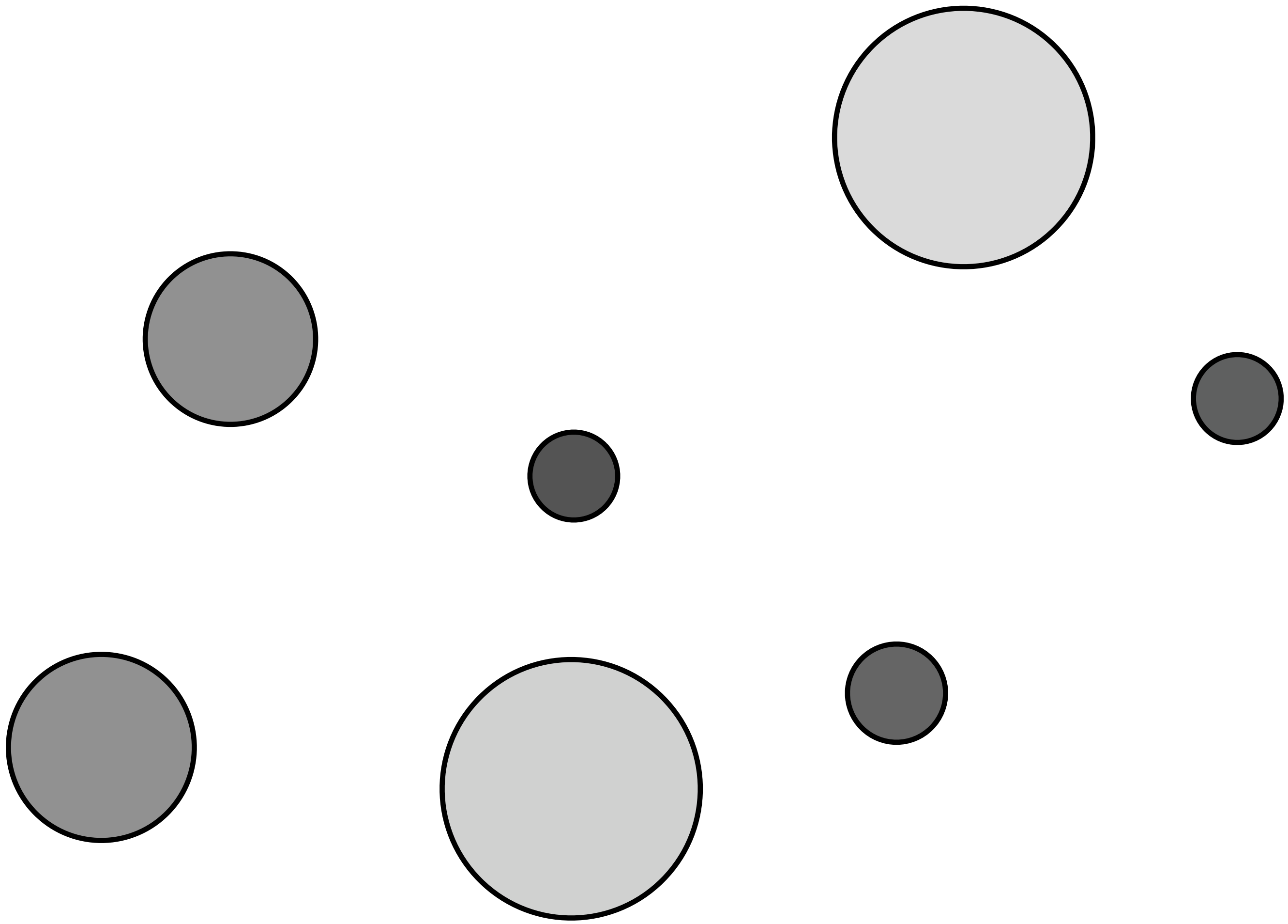


Lighter color: higher growth rate

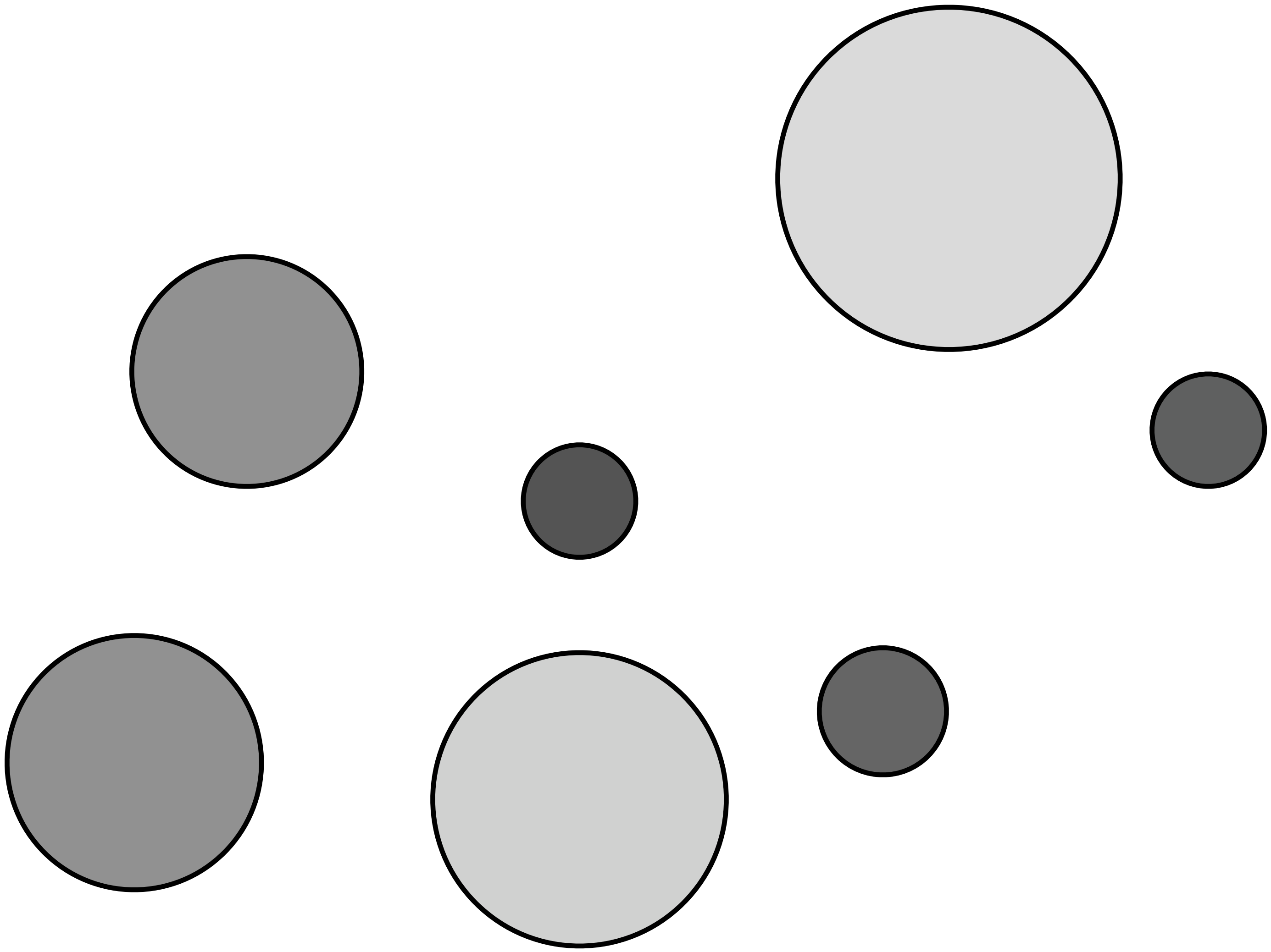
Cartoon of model



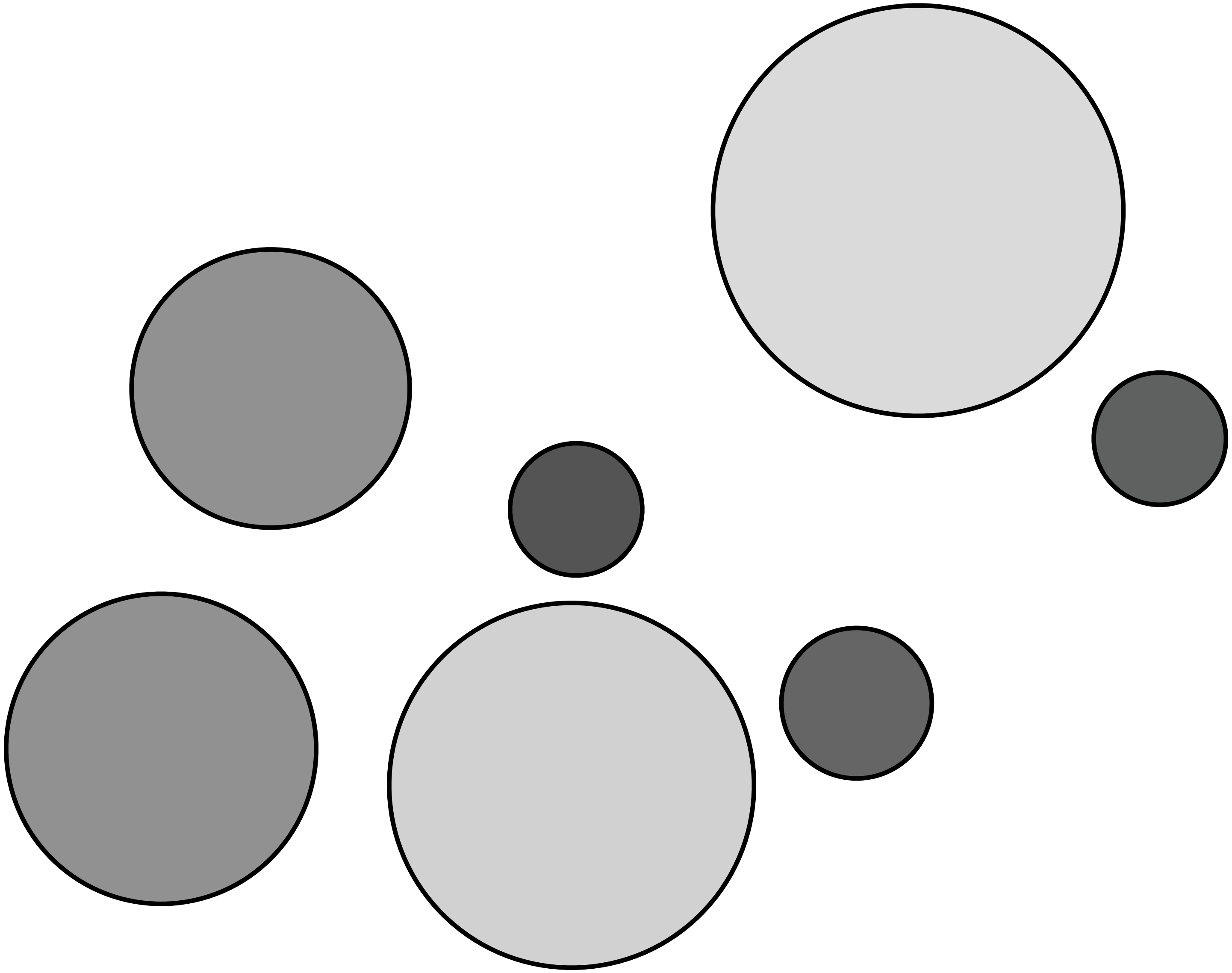
Cartoon of model



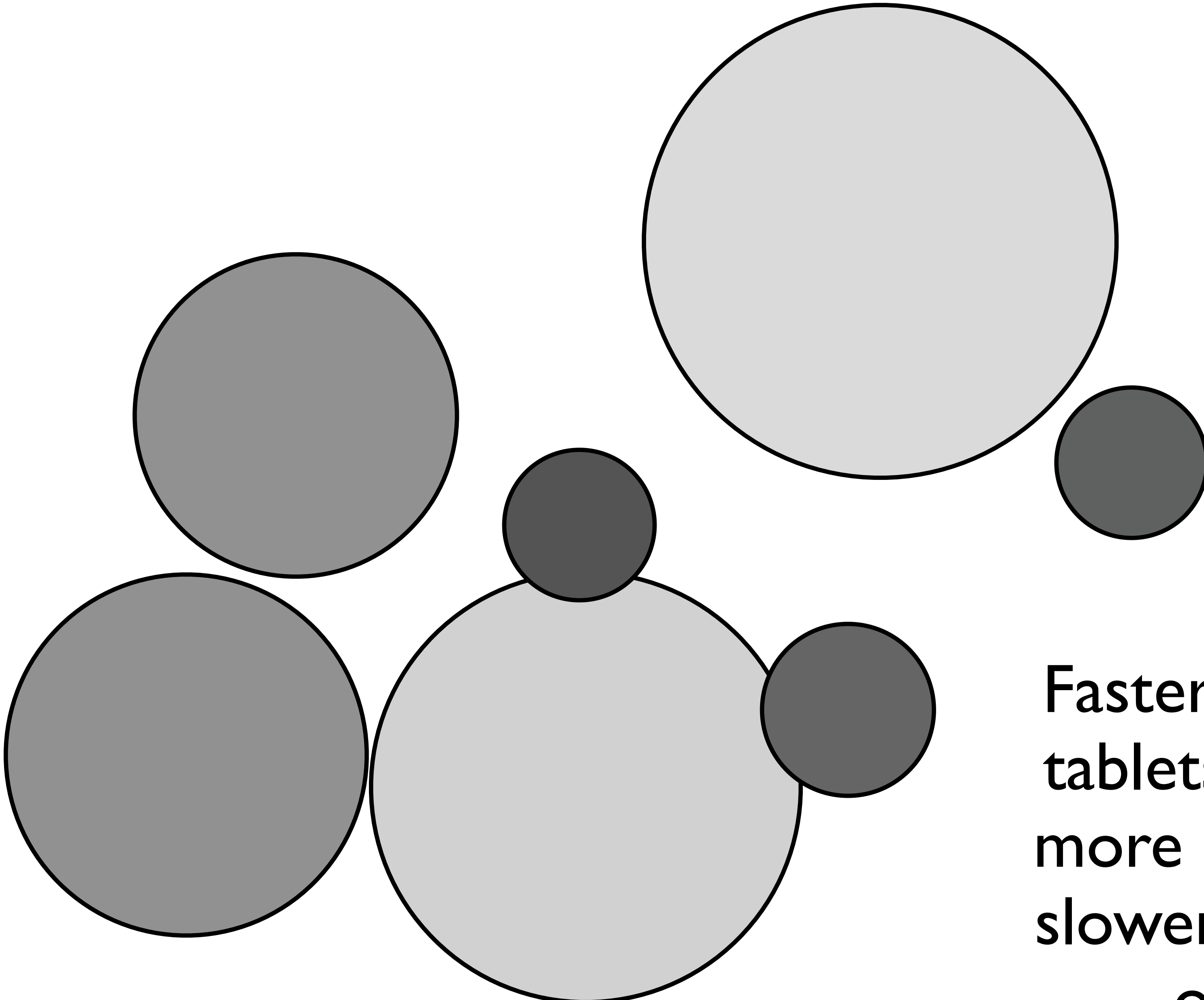
Cartoon of model



Cartoon of model



Cartoon of model



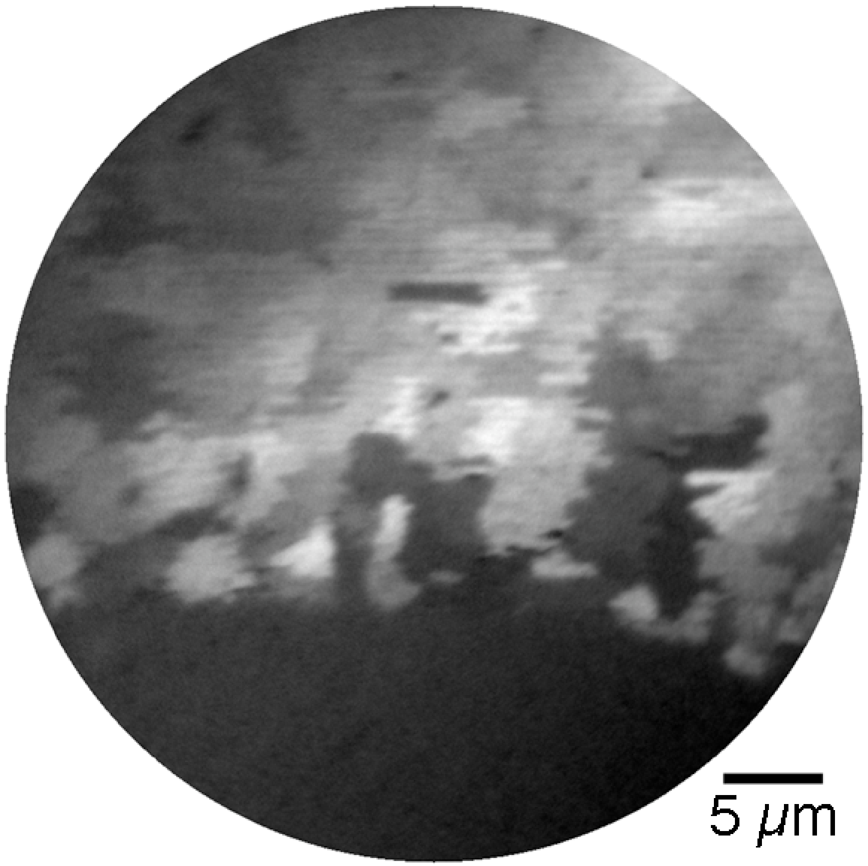
Faster-growing tablets take up more area than slower-growing ones.

Model for orienting nacre tablets is closely related to mutation-selection models in population biology

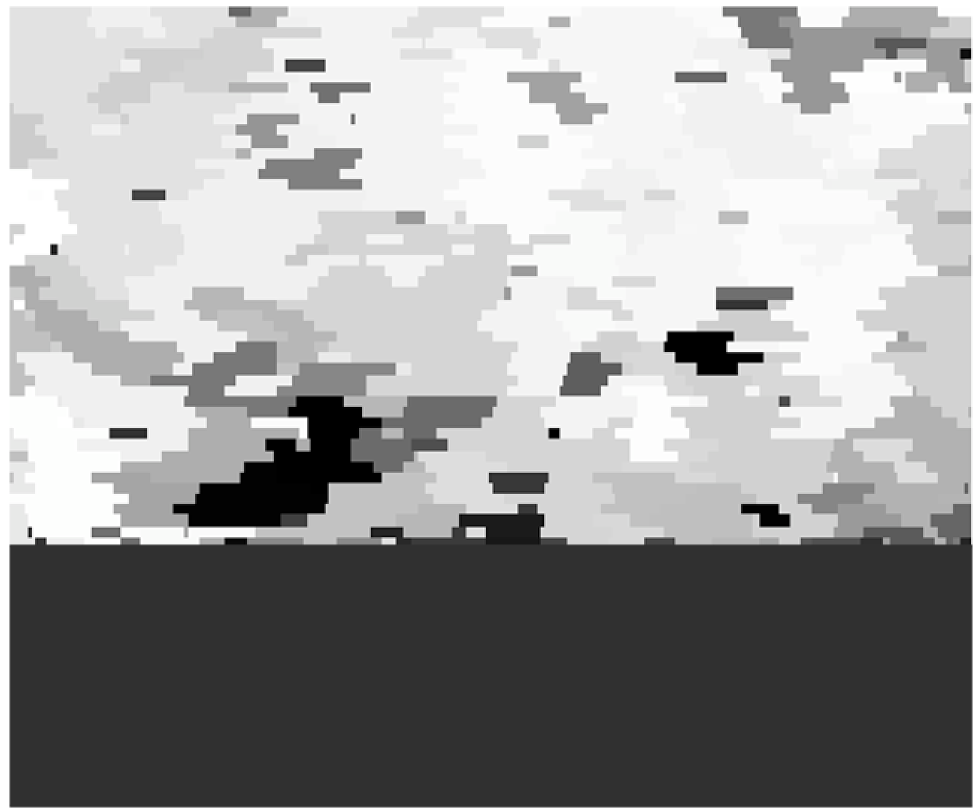
Nacre growth	Population biology
Oriented tablets grow the fastest	Organisms with higher fitness reproduce the fastest
Misoriented tablets occur occasionally	Sequence changes from mutations occur occasionally

M. Turelli, Theor. Popul. Biol. 25, 138 (1984); S.N. Coppersmith, R.D. Blank, and L.P. Kadanoff, J. Stat. Phys. 97, 429 (1999)

Results for simple model look remarkably like experiment

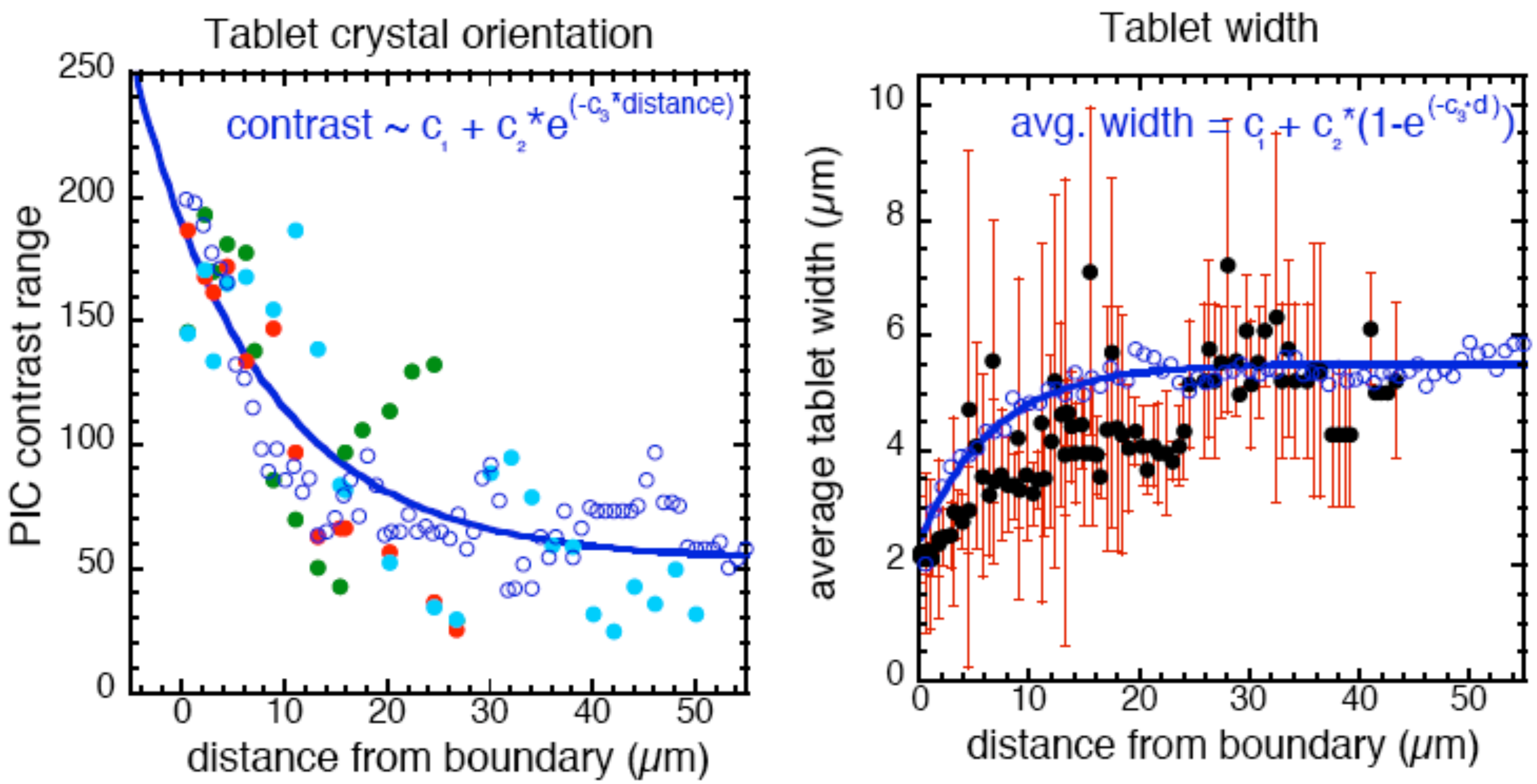


Experimental data



Model
ϵ=0.028, δ=0.2

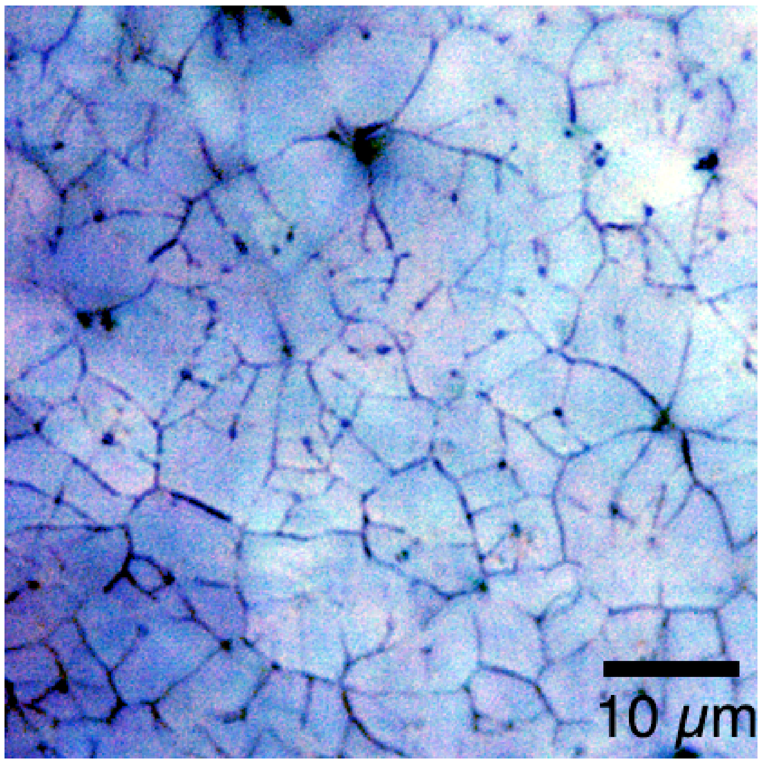
Model is consistent with measured variation of c-axis alignment and with evolution of tablet widths



Same parameters are used for both panels: ϵ=0.025, δ=0.3

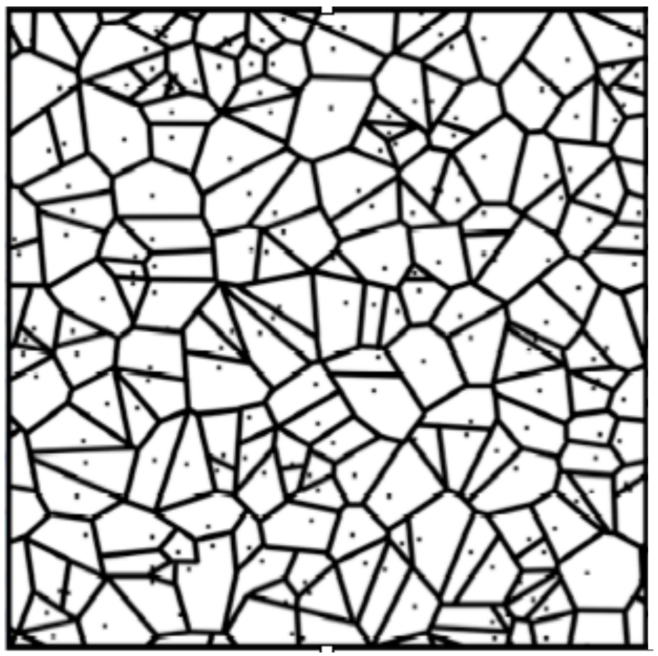
Growth model with unequal growth rates yields curved tablet boundaries, consistent with experiment

abalone nacre tiles
top view
polished, imaged with VLM-DIC
1000x with Zeiss Axiotech 100

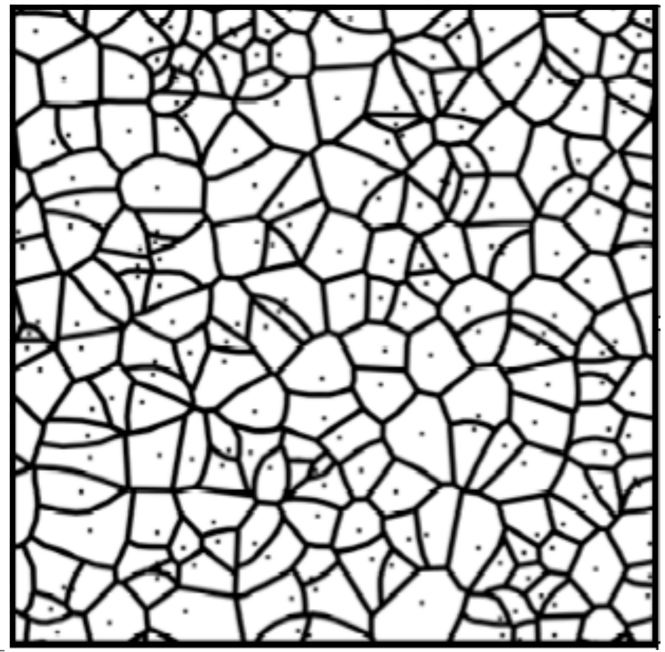


model configurations

δ=0 (straight boundaries)

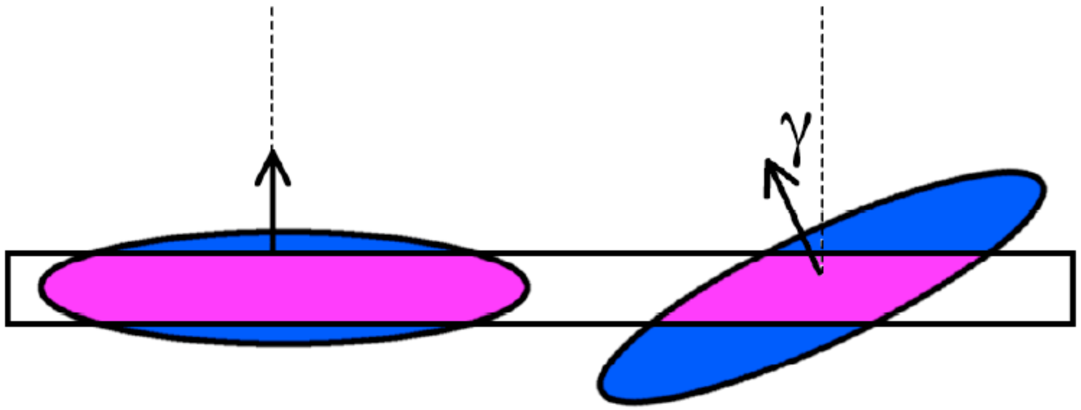


δ=0.3 (curved boundaries)



P. Gilbert, et al., JACS, accepted for publication

Proposed mechanism for having fastest growth for tablets oriented with (001) perpendicular to layers: suppression of crystal growth rate along (001) direction



- If crystals grow faster in a-b plane than along c axis, then tablets with c-axis perpendicular to layers grow the fastest and “crowd out” slower-growing misaligned ones

Nacre proteins are known to suppress aragonite growth rate along (001)

L. Addadi et al., PNAS 82, 4110 (1985)

Model is consistent with:

- establishment of orientational alignment over tens of microns
- evolution of widths of regions with same crystal orientation
- curved tablet boundaries
- previous observations of suppression of c-axis growth rate by nacre proteins

Upshot: organism controls crystal orientation indirectly, via control of crystal nucleation and growth rates

Summary

- Synchrotron spectromicroscopy provides unprecedented information about the organization of nacre.
- Coordinating experiment and theory enables substantial progress in understanding the physical mechanisms that give rise to nacre’s highly organized and optimized structure.

The future

- Work to understand why orienting aragonite tablets is desirable — does it help toughness?
- Use spectromicroscopy to investigate other biomaterials
- Use theoretical modeling to gain understanding into the physical processes that give rise to the observed structures
- Use insight into formation mechanisms to understand the design principles exploited by biological systems to create biomaterials with highly optimized properties

Acknowledgments

Collaborators:

- Rebecca Metzler, Ronke Obalisi, Mike Abrecht, Andrew Konicek, David Grierson, Dong Zhou, P.U.P.A. Gilbert

Funding: NSF, DOE

References: R. Metzler et al., Phys. Rev. Lett. **98**, 268102 (2007)
R. Metzler et al., Phys. Rev. B **77**, 064110 (2008)
P. Gilbert et al., J. Am. Chem. Soc. **130**, 17519-17527, 2008.