Colloid assembly and transformation (CAT): The relationship of PILP to biomineralization

Laurie Gower*, Jeremy Elias

Department of Materials Science & Engineering, University of Florida, Gainesville, FL, USA

A R T I C L E   I N F O
Edited by Bauke W. Dijkstra

Keywords:
Biominalization
PILP
Colloid assembly and transformation (CAT)
Intrinsically disordered proteins (IDPs)
Non-classical crystallization
Amorphous precursor

A B S T R A C T

The field of biominalization has undergone a revolution in the past 25 years, which paralleled the discovery by Gower of a polymer-induced liquid-precursor (PILP) mineralization process. She proposed this in vitro model system might be useful for studying the role biopolymers play in biominalization; however, the ramifications of this pivotal discovery were slow to be recognized. This was presumably because it utilized simple polypeptide additives, and at that time it was not recognized that the charged proteins intimately associated with biominalizers are often intrinsically disordered proteins (IDPs). Over the years, many enigmatic biominal features have been emulated with this model system, too many to be mere coincidence. Yet the PILP system continues to be underacknowledged, probably because of its namesake, which indicates a “liquid precursor”, while we now know this phase appears to have viscoelastic character. Another factor is the confusing semantics that arose from the discovery of multiple “non-classical crystallization” pathways. This review suggests a more relevant terminology for the polymer-modulated reactions is “colloid assembly and transformation (CAT)”, which we believe more accurately captures the key stages involved in both biominalization and the PILP process. The PILP model system has helped to decipher the key role that biopolymers, namely the IDPs, play in modulating biominalization processes, which was not readily accomplished in living biological systems. Some remaining challenges in understanding the organic–inorganic interactions involved in biominalization are discussed, which further highlight how the PILP model system may prove invaluable for studying the simple, yet complex, CAT crystallization pathway.

1. Introduction

A polymer-induced liquid-precursor (PILP) mineralization process was discovered by Gower ~25 years ago (Gower and Odom, 2000). However, this PILP discovery, even though it was distinctly different from the classical crystallization processes that were summarized at the beginning of every biominal review, went largely unnoticed until the Gower group gradually demonstrated that this PILP model system could emulate many of the enigmatic features of biominalizers that pervaded the 1990s literature (Gower, 2008; Amos et al., 2006). Starting with non-equilibrium morphologies, the hallmark of invertebrate biominalizers, to interpenetrating nanostructured composites (Fig. 1); but perhaps even more revealing is the similar defect textures (Fig. 2), because ‘mineralogical signatures’ point to crystallization mechanisms, which we now know follow a non-classical pathway (De Yoreo et al., 2015). Yet even with this ability to emulate so many features, which surely could not be coincidence, the community still rarely refers to biominalization as occurring through a PILP-like process. The reason for this, we believe, is because the PILP namesake includes the word “liquid”, yet it has become clear in recent years that the amorphous precursor phase of biominalizers is not a pure liquid phase given that biominalizers ubiquitously have a remnant colloidal or nanogranular texture (Fig. 2B) (De Yoreo et al., 2015). On the other hand, the biominal precursor phases are presumably not solid particles given their complete space-filling properties. A recent paper suggests that solid particles attach and then the remaining space is filled in by ion-by-ion growth (Sun et al., 2020). This seems unlikely given that one would expect diffusion-based filling in to occur from outside-inward, which would soon block entry and leave large amounts of porosity in the interior. While entrapped pores are occasionally seen, most biominalizers are fully densified. Therefore, this paper is intended to clarify where we believe such incongruities arise, and to propose a description that more adequately captures what is occurring in biominalization, as derived from our experiences with the PILP model system.
2. Consistency of precursor phase

One might ask, what is wrong with the terminology that has come into play in recent years, namely “particle attachment” (De Yoreo et al., 2015). Firstly, we have noticed that people seem to interchangeably refer to “particle attachment” as “oriented attachment”, and they are not at all the same. Oriented attachment is just one of several particle attachment scenarios, and for ACC precursors, it’s not even relevant as...
there is no crystallographic order that would require orienting to match with neighboring particles. Secondly, the word “particles” implies a solid phase, which does not appear to be the case. In the PILP model system, as well as the particle accretion process in biominerals, whose mineralogical signatures are equivalent (Fig. 2), the densified textures suggest the accumulating precursor phase probably has a viscoelastic consistency at the time it is being molded. In other words, a viscous consistency that can flow over time could provide the gradual coalescence that leads to densified, space filling properties, but while also leaving a remnant colloidal texture from the particles/droplets being coated with polymer. Recent cryo-TEM studies of the PILP phase show what appears to be nanogranular fluid (Xu et al., 2018), where the non-homogeneous phase was described as consisting of ~2 nm-sized ACC clusters (Fig. 3A). Other groups have considered these subunits to be pre-nucleation clusters (PNCs), a subject of extensive debate (Gebauer et al., 2014). Regardless of the terminology, one could envision that a “fluid” comprised of PNC nanograins intercalated with polymer could explain the unusual flow behavior of the PILP phase, such as why streams of PILP phase only slowly coalesce over time (Fig. 3B&C). Likewise, a viscoelastic consistency could explain the gradual densification of “particles” that attach during biomineralization. Even the original PILP papers described the liquid-like character of the PILP phase as being short-lived (Gower and Odom, 2000; Kim et al., 2007), where in minutes it densified to where droplets only partially coalesced, and deposited films became solids with various consistencies before ultimately undergoing the amorphous-to-crystalline transformation (Fig. 3 D-I). These observations suggest the viscoelastic tendency of the phase arises from some type of non-covalent crosslinking. Reversible crosslinking could be ion-based, such as calcium-mediated crosslinks with the intercalated polymer chains; or possibly even a hydrogen-bonding network could be created in these densified phases from interactions between polymer and hydrogenated carbonates (or phosphates in the calcium phosphate system).

3. Biominal textures

In both the PILP system and biominerals, there is a remnant nanoscale texture created by the accretion of precursor colloids (Fig. 2B) (Gower, 2008; De Yoreo et al., 2015; Kim et al., 2007; Sethmann et al., 2005; Gal et al., 2014; Gilbert et al., 2019). This nanogranular texture would be expected to be enhanced by the exclusion of the polymeric impurities during crystallization, which indeed has been shown to be the case in the nanograins in mollusk nacre (Fig. 2B) (Rousseau et al., 2005). Not only should one expect polymer be enriched at the surface of the colloids, given that it is involved in creating/stabilizing the phase, one would also expect polymer (and Mg and other impurities) to be excluded during crystallization. Indeed, when the PILP precursor undergoes the amorphous-to-crystalline transformation, exclusion becomes spatially limited, leading to “transition bars” in the PILP forming tablets (Gower and Odom, 2000; Dai et al., 2008), which coincidentally match the etching patterns seen in nacre (Fig. 2A) (Weedon and Taylor, 1995). This leads to occlusion of organics to form mesocrystals (i.e. mesoskopically structured crystal composed of numerous crystallographically aligned nanocrystals that are spatially separated), or polymer might be excluded to “grain” boundaries (e.g. edges of nacre tablets and interlamellar sheets) (Fig. 2A-C), etc, leading to “fuzzy” interfaces with interesting mechanical properties.

Layering is also ubiquitous in pathological deposits, such as the concentric laminations in kidney stones and Randall’s plaques (Evan et al., 2006) (Fig. 2C). Ex vivo observations of biominerals have often led to the conclusion that organics at phase boundaries were indicative of their function as crystal growth modifiers, or that they provided a pre-formed compartment, or they were daily growth rings, etc, whereas in reality, many occluded organics might simply be excluded impurities (Gower et al., 2010; Amos et al., 2009). Nearly all biominerals have mesoscale layering (Cuif et al., 2012). This is not only important with respect to mineralogical ‘signatures’ of formation mechanism but is also of great interest toward understanding biomineral’s remarkable mechanical properties. Much literature has focused on the enhanced
fracture toughness that such layering provides, but the layering is usually assumed to result from cellular control. Evolutionary selection would include both mesoscale textures created by this formation mechanism as well as deliberate cell-controlled microstructures.

Gower’s Chem. Review paper in 2008 provided an extensive discussion on these features (Gower, 2008). Notably, that paper is 13 years old, and yet as the field continues to employ more advanced characterization tools, the findings always parallel what has already been observed with the PILP model system.
Intrinsically disordered proteins (IDPs) as process-directing agents

It has become clear over the years that one reason the PILP model system didn’t gather the attention it deserved was because it was an exceedingly simple in vitro model system that used polymer additives that were not real proteins. Back in the 1990s, one simply did not expect a repetitive polypeptide such as polyaspartic acid to be able to mimic the functionality of a real protein. At that time, people considered proteins to be fibrous or globular, whose form and function were based on the classic “lock-and-key” mechanism of molecular recognition. Therefore, the biominalization hypotheses were focused on demonstrating a high degree of specificity between the functional groups on a protein surface that might have an epitaxial or stereospecific relationship with the crystal lattice (Addadi et al., 1989). But it is now recognized that many proteins, especially those that are highly charged (as are the biominal proteins!), are intrinsically disordered proteins (IDPs) (Wojtas et al., 2012). Thus, they take the form of a random coil polyelectrolyte, as does polyanionic acid, etc. One can now see why this overly simple model system was capable of emulating many of the morphological features of biominerals. That’s not to say that IDPs do not have domains with specific interactions with ion clusters or crystal surfaces, and certainly they do with cell integrins, but the role IDPs play in morphological control of biominerals clearly falls in the realm of materials science – polyelectrolytes sequester ion clusters that phase separate in solution. Thus, from this perspective, the role of these charged biopolymers, namely the IDPs, seems to be a process-directing agent (as Gower proposed years ago) (Gower and Odom, 2000; Thula et al., 2011), in a process that the community now defines as a non-classical crystallization process. But with the growing number of non-classical pathways suggested in that Science paper (De Yoreo et al., 2015), we propose the CAT mechanism may a better terminology for describing the key role such IDP biopolymers play in biominalization, as illustrated in Fig. 5.

6. Paleontology simplified

Was it any surprise that Gilbert’s group found evidence of “particle attachment” across many phyla in the paleontological record (Gilbert et al., 2013)? As Gower suggested some years ago (Gower and Odom, 2000; Gower and Tirrell, 1998), there’s a reason that nacre in mollusks and semi-nacre in bryozoans, which are in organisms that evolved down very different evolutionary paths, both exhibit a similar thin tablet morphology (Gower, 2008). That same morphology was readily reproduced with the PILP model, and such a thickness was simply a result of
a given supersaturation of calcium carbonate that can be sequestered and stabilized by polymer in the media without crashing out in an uncontrolled fashion. Certainly, the first step in the evolution of biominerals had to be in biopolymer sequestration of calcium to avoid cell toxicity, and a more soluble phase provides ease of ion mobility for homeostasis. The IDPs likely evolved from sequestering ions away to...
Fig. 4. Non-classical crystallization pathways and significance of CAT to biomineralization. (Left) A variety of non-classical crystallization pathways have been resolved in recent years, which firstly demonstrates the power of in vitro model systems. The pathway most relevant to biomineralization is arguably the liquid droplet pathway. However, even though a liquid condensed phase exists without polymer, without polymer additive, the result would simply be the euhedral “Bulk crystal” represented as the final product. (Right) An adaptation of this popular schematic shows the “Droplets” pathway takes a dramatically different turn when polymer is added, as discovered by the PILP system. The liquid condensed phase is sequestered in larger quantity with polymer, and most importantly, is stabilized long enough to enable the pseudomorphic transformation. This provides a means for delivering the hallmark of biominerals, the molding of species-specific non-equilibrium morphologies. The second hallmark of biominerals is their mesocrystalline texture, which has also been demonstrated with the PILP model system. Occluded organics may be organized along specific crystallographic planes due to the symmetric exclusion of impurities into the transition bars, or it could become entrapped randomly, and especially concentrated at grain boundaries and phase boundaries, such as between temporally deposited secretions of precursor. The third hallmark of biominerals is the interpenetrating composites that are created through infiltration of the precursor phase into organized matrices. Although the mechanism of infiltration remains controversial, it does not occur without polymer additive, and some type of precursor phase is visualized both in vitro and in vivo (in bone). Given that infiltration would not be expected to occur with solid particles (which are sometimes visualized in cryo-EM as being much too large to fit within the narrow confines of collagen fibrils), there apparently is some ‘fluidic’ quality to this precursor phase that allows its infiltration into matrices, which Gower’s group proposed occurs through capillarity. Adapted reprint from De Yoreo et al., 2015, with permission from AAAS.

7. Continuing value of the PILP in vitro model system for studying CAT

With the conceptual premise of CAT, the key questions then become, how do the biopolymers collect ions and stabilize the amorphous colloids long enough for them to transform via pseudomorphic transformation, thereby yielding species-specific single crystals with molded non-equilibrium morphologies? This usually occurs in some type of mineral deposition vesicle or syncytium (Beniash et al., 1997). Alternatively, how do the precursor colloids interact with fibrous matrices, collagen in vertebrate bone (Olszta et al., 2007), chitin in invertebrate exoskeletons, to infiltrate into the interstices and yield interpenetrating composites? We know a lot about these self-organizing matrices and compartments, but how they interact with and modulate the assembly of the precursor colloids is really the next stage (in our minds) of solving the biomineralization puzzle.

On the inorganic side, while the PILP process seems like a simple physicochemical process of polyelectrolyte interaction with ion clusters, there are still challenges in capturing and measuring the properties of the PILP phase, particularly as it is a dilute and dynamically changing system, both in composition and consistency (Kim et al., 2007; Dai et al., 2008). One can imagine that in vivo, by the time such a phase is transported to where it is forming the biomineral, it will have likely densified into a viscoelastic liquid or gel (as observed in model systems via in situ AFM (Wolf et al., 2017), and/or these changes in consistency may very well be influenced by its interactions with the substrate or matrix (Kim et al., 2007), as well as the IDP processing-directing agent. That PILP macropod under the bubble (Fig. 3B) never did crystallize (Gower and Odom, 2000), suggesting the thickness of precursor can be an issue. Confinement is known to stabilize ACC (Stephens et al., 2010), perhaps because impurities (polymer, water) can’t be effectively removed; or the proper ratio of counterions can’t be achieved; or the bicarbonate/carbonate ratio may not be adequate. Unlike many studies on calcium carbonate, most of the PILP experiments were done at a neutral pH, where bicarbonates are the dominant species. This may contribute to the inhibitory action in preventing classical nucleation, and as mentioned earlier, they might also provide a hydrogen bonding network that creates viscoelastic character of the phase. In other words, there are many more features that need to be studied, and clearly the biological aspects which haven’t been addressed here are important with respect to the spatiotemporal secretion of matrices and mineral processing additives, but we believe that a further mechanistic understanding of the physicochemical processes involved in biomineralization can best be accomplished with a clean in vitro model system, such as the PILP system, which allows for in situ advanced characterization of CAT processes.
CRediT authorship contribution statement

Laurie Gower: Conceptualization, Supervision, Project administration, Funding acquisition, Resources, Methodology, Visualization, Writing – original draft. Jeremy Elias: Investigation, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
shown here, the A-to-C transformation might occur through a random sporadic pathway across connected bits of precursor phase (as seen in mollusk nacre), or via crystal to have the same crystallographic orientation across bends and curves as it is molded into its species-defined non-equilibrium morphology. Although not more pronounced in the densely-packed matrices of collagen and chitin, whose fibrils template the orientation of hydroxyapatite nanocrystals in bone and dentin, or the chitin-guided orientation of calcium carbonate/phosphate in crustacean cuticles. In these dense matrices, the organic matrix is roughly 50 vol%, which yields interpretative nanocomposites with remarkable mechanical properties. (A) A pseudomorphic transformation proceeds the pathway from liquid-solid to solid-solid for the entire crystal to have the same crystallographic orientation across bends and curves as it is molded into its species-defined non-equilibrium morphology. Although not shown here, the A-to-C transformation might occur through a random sporadic pathway across connected bits of precursor phase (as seen in mollusk nacre), or via spherical growth or plume-like splay across channels (as in corals), or it might follow along step-edges of crystallographic symmetry, similar to classical crystallization where growth spirals around dislocation ledges. In the latter case, one might expect the impurity proteins to be excluded and thus occluded along well-defined crystalline planes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Acknowledgments

This review is based on Gower’s groups over the years who have contributed to multiple projects throughout her career, with the data presented in this review primarily resulting from the following National Science Foundation (NSF) grants BES-9980795, DMR-0710605, DMR-0994209, DMR-1309657 and the National Institutes of Health (NIH) grants NIDDK R01 DK59765, NIDDK R01DK092311, NIDCR R01 DE018649.

References:

Gower, L.B., Odum, D.J., 2000. Deposition of calcium carbonate films by a polymer-induced liquid-precursor (PILP) process. J. Crystal Growth 210 (4), 719–734.

Gower, L.B., 2008. Biomimetic model systems for investigating the amorphous precursor pathway and its role in biomineralization. Chem. Rev. 108 (11), 4551–4627.

Amos, F.F., Olszta, M.J., Khan, S.R., Gower, L.B., 2006. Relevance of a polymer-induced liquid-precursor (PILP) mineralization process to normal and pathological biomineralization. In: Konigsberger, E., Konigsberger, L. (Eds.), Biomimetic–Bioengineering–Medical Aspects of Solubility. John Wiley & Sons Ltd, West Sussex, England, pp. 125–217.

De Voreo, J.J., Gilbert, P.U.P.A., Sommerdijk, N.A.J.M., Putnis, A., Weis, J., 2009. Evidence on two major concepts in invertebrate biomineralization studies. Minerals 9 (2582), 1.

Kim, Y.-Y., Douglas, E.P., Gower, L.B., 2007. Patterning inorganic (CaCO3) thin films via a polymer-induced-liquid-precursor process. Langmuir 23 (9), 4862–4870.

Sethmann, L., Putnis, A., Grassmann, O., Lobmann, P., 2005. Observation of nano-clustered calcite growth via a transient phase mediated by organic polymers: A close match for biomineralization. Am. Mineral. 90, 1213–1217.

Gal, A., Kahil, K., Vidavsky, N., DeVol, R.T., Gilbert, P., Fratzl, P., Weiner, S., Addadi, L., 2014. Particle accretion mechanism underlies biological crystal growth from an amorphous precursor phase. Adv. Funct. Mater. 24 (34), 5420–5426.

Gower, L.B., Amos, F.F., Khan, S.R., Gower, L.B., 2004. Nanofibrous calcite synthesized via a solution-precursor-solid mechanism. Crystal Growth & Design 16 (12), 2355–2362.

Rousseau, M., Lopera, E., Stemple, P., Brendle, M., Franco, E., Guette, A., Naskai, R., Bouzam, X., 2005. Multiscale structure of sheet nacre. Biomaterials 26 (31), 6254–6262.

Dai, L., Cheng, X., Gower, L.B., 2008. Transition bars during transformation of an amorphous calcium carbonate precursor. Chem. Mat. 20 (22), 6917–6928.

Weedon, M.J., Taylor, P.D., 2008. Crystal assembly in bone: implications for comparative biomineralization of lophophorates and molluscs. Biol. Bull. 188, 281–292.

Evans-Lutterodt, K., Nutt, S.R., Zavattieri, P., Kisailus, D., 2014. Bio-inspired impact- and fatigue-stable materials: Design 16 (2), 153–160.

Cheng, X., Gower, L.B., 2009. Biominetic synthesis of calcite films by a polymer-induced liquid-precursor (PILP) process 1. Influence and incorporation of magnesium. J. Crystal Growth 307 (2), 496–504.

Cuif, J.P., Dauphin, Y., Nehrke, G., Noutet, J., Perez-Huerta, A., 2012. Layered growth and crystallization in calcareous biominerals: impact of structural and chemical evidence on two major concepts in invertebrate biomineralization studies. Minerals 5 (3), 11–39.

Gower, L.A., Tirrell, D.A., 1998. Calcium carbonate films and helices grown in solutions synthesized via a solution-precursor-solid mechanism. Crystal Growth & Design 16 (12), 2355–2362.

Rousseau, M., Lopera, E., Stemple, P., Brendle, M., Frange, A., Naskai, R., Bouzem, X., 2005. Multiscale structure of sheet nacre. Biomaterials 26 (31), 6254–6262.

Dai, L., Cheng, X., Gower, L.B., 2008. Transition bars during transformation of an amorphous calcium carbonate precursor. Chem. Mat. 20 (22), 6917–6928.

Weedon, M.J., Taylor, P.D., 2008. Crystal assembly in bone: implications for comparative biomineralization of lophophorates and molluscs. Biol. Bull. 188, 281–292.

Evans-Lutterodt, K., Nutt, S.R., Zavattieri, P., Kisailus, D., 2014. Bio-inspired impact- and fatigue-stable materials: Design 16 (2), 153–160.

Cheng, X., Gower, L.B., 2009. Biominetic synthesis of calcite films by a polymer-induced liquid-precursor (PILP) process 1. Influence and incorporation of magnesium. J. Crystal Growth 307 (2), 496–504.

Cuif, J.P., Dauphin, Y., Nehrke, G., Noutet, J., Perez-Huerta, A., 2012. Layered growth and crystallization in calcareous biominerals: impact of structural and chemical evidence on two major concepts in invertebrate biomineralization studies. Minerals 5 (3), 11–39.

Gower, L.A., Tirrell, D.A., 1998. Calcium carbonate films and helices grown in solutions synthesized via a solution-precursor-solid mechanism. Crystal Growth & Design 16 (12), 2355–2362.

Rousseau, M., Lopera, E., Stemple, P., Brendle, M., Frange, A., Naskai, R., Bouzem, X., 2005. Multiscale structure of sheet nacre. Biomaterials 26 (31), 6254–6262.

Dai, L., Cheng, X., Gower, L.B., 2008. Transition bars during transformation of an amorphous calcium carbonate precursor. Chem. Mat. 20 (22), 6917–6928.

Weedon, M.J., Taylor, P.D., 2008. Crystal assembly in bone: implications for comparative biomineralization of lophophorates and molluscs. Biol. Bull. 188, 281–292.

Evans-Lutterodt, K., Nutt, S.R., Zavattieri, P., Kisailus, D., 2014. Bio-inspired impact- and fatigue-stable materials: Design 16 (2), 153–160.

Cheng, X., Gower, L.B., 2009. Biominetic synthesis of calcite films by a polymer-induced liquid-precursor (PILP) process 1. Influence and incorporation of magnesium. J. Crystal Growth 307 (2), 496–504.

Cuif, J.P., Dauphin, Y., Nehrke, G., Noutet, J., Perez-Huerta, A., 2012. Layered growth and crystallization in calcareous biominerals: impact of structural and chemical evidence on two major concepts in invertebrate biomineralization studies. Minerals 5 (3), 11–39.

Gower, L.A., Tirrell, D.A., 1998. Calcium carbonate films and helices grown in solutions synthesized via a solution-precursor-solid mechanism. Crystal Growth & Design 16 (12), 2355–2362.

Rousseau, M., Lopera, E., Stemple, P., Brendle, M., Frange, A., Naskai, R., Bouzem, X., 2005. Multiscale structure of sheet nacre. Biomaterials 26 (31), 6254–6262.

Dai, L., Cheng, X., Gower, L.B., 2008. Transition bars during transformation of an amorphous calcium carbonate precursor. Chem. Mat. 20 (22), 6917–6928.

Weedon, M.J., Taylor, P.D., 2008. Crystal assembly in bone: implications for comparative biomineralization of lophophorates and molluscs. Biol. Bull. 188, 281–292.

Evans-Lutterodt, K., Nutt, S.R., Zavattieri, P., Kisailus, D., 2014. Bio-inspired impact- and fatigue-stable materials: Design 16 (2), 153–160.

Cheng, X., Gower, L.B., 2009. Biominetic synthesis of calcite films by a polymer-induced liquid-precursor (PILP) process 1. Influence and incorporation of magnesium. J. Crystal Growth 307 (2), 496–504.

Cuif, J.P., Dauphin, Y., Nehrke, G., Noutet, J., Perez-Huerta, A., 2012. Layered growth and crystallization in calcareous biominerals: impact of structural and chemical evidence on two major concepts in invertebrate biomineralization studies. Minerals 5 (3), 11–39.
Williams, R.J.P. (Eds.), Biomineralization - Chemical and Biochemical Perspectives. VCH Pub, N. Y., pp. 133–156.

Wingender, B., Bradley, P., Saxena, N., Ruberti, J., Gower, L., 2016. Biomimetic organization of collagen matrices to template bone-like microstructures. Matrix Biol. 52-54, 384–396.

Wojtas, Magdalena, Dobryszycki, Piotr, Oyhar, Andrej, 2012. Intrinsically disordered proteins in biomineralization. In: Seto, Jong (Ed.), Advanced Topics in Biomineralization. InTech.

Thula, T.T., Svedlund, F., Rodriguez, D.E., Podschun, J., Pendi, L., Gower, L.B., 2011. Mimicking the nanostructure of bone: comparison of polymeric process-directing agents. Polymers 3 (1-3), 10–35.

Amos, F.F., Sharbaugh, D.M., Talham, D.R., Gower, L.B., 2007. Formation of single-crystalline aragonite tablets/films via an amorphous precursor. Langmuir 23 (4), 1988–1994.

Benias, E., Aizenberg, J., Addadi, L., Weiner, S., 1997. Amorphous calcium carbonate transforms into calcite during sea urchin larval spicule growth. Proc. R. Soc. Lond. B 264 (1380), 461–465.

Lo, Y.H., Zhou, J., Rana, A., Morrill, D., Gentry, C., Enders, B., Yu, Y.-S., Sun, C.-Y., Shapiro, D.A., Falcone, R.W., Kapteyn, H.C., Murzane, M.M., Gilbert, P.U.P.A., Miao, J., 2021. X-ray linear dichroic ptychography. Proc. Natl. Acad. Sci. 118 (2) e2019068118.

Masic, A., Weaver, J.C., 2015. Large area sub-micron chemical imaging of magnesium in sea urchin teeth. J. Struct. Biol. 189 (3), 269–275.

Otsuka, M.J., Cheng, X., Lee, S.S., Kuntz, R., Kim, Y.-Y., Kaufman, M.J., Douglas, E.P., Gower, L.B., 2007. Bone structure and formation: A new perspective. Mater. Sci. Eng. R-Rep. 58 (3-5), 77–116.

Dai, L., Douglas, E.P., Gower, L.B., 2008. Compositional analysis of a polymer-induced liquid-precursor (PILP) amorphous CaCO$_3$ phase. J. Non-Crystall. Solids 354 (17), 1845–1854.

Wolf, S.J.P., Caballero, L., Meo, F., Colfen, H., 2017. Gel-like calcium carbonate precursors observed by in situ AFM. Langmuir 33 (1), 158–163.

Stephen, C.J., Ladden, S.F., Meldrum, F.C., Christenson, H.K., 2010. Amorphous calcium carbonate is stabilized in confinement. Adv. Funct. Mater. 20 (13), 2108–2115.