Melanin and Glycera Jaws

EMERGING DARK SIDE OF A ROBUST BIOCOMPOSITE STRUCTURE*

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Defining the design principles guiding the fabrication of superior bio composite structures from an assemblage of ordinary molecules is a key goal of biomimetics. Considering their low degree of mineralization, Glycera jaws have been shown to be extraordinarily resistant to abrasion based on the metric hardness\(^3/\)Young’s modulus\(^2\). The jaws also exhibit an impressive chemical inertness withstanding boiling concentrated hydrochloric acid as well as boiling concentrated sodium hydroxide. A major organic component largely responsible for the chemical inertness of the jaws has been characterized using a spectrophotometric assay for melanin content,\(^{13}\)C solid state nuclear magnetic resonance, IR spectroscopy, and laser desorption ionization-time of flight mass spectrometry and is identified here as a melanin-like network. Although melanin is widely distributed as a pigment in tissues and other structural biomaterials, to our knowledge, Glycera jaws represent the first known integument to exploit melanin as a cohesive load- and shape-bearing material.

Nature is richly endowed with blueprints for building robust load-bearing structures from ordinary biomolecules such as minerals, proteins, polysaccharides, and polymers of secondary metabolites. The architecture and general composition of the jaws of Glycera species have been studied for decades (1–4), but their precise chemical structure remains unclear. Lichtenegger et al. (4) recently reported that Glycera jaws contain polycrystalline nanofibers of the copper hydroxychloride atacamite (Cu\(_2\)Cl(OH)\(_3\)). This discovery was of particular significance as it posed to have mechanical consequences in insect cuticle (14, 15). One of these functions, protein cross-linking, was proposed to have mechanical consequences in insect cuticle (14, 16, 17); changes in the mechanical properties of fungal cell walls have also been correlated with melanin synthesis (18). Melanins can be crudely classified as black eumelanins and yellow-to-brown pheomelanins, and in plants, fungi, and bacteria, as brown-to-black allomelanins (7). Eumelanins generally consist of a network of about 4–6 covalently linked indole groups per layer, sometimes functionalized with carboxylates, hydroxyls, or other groups (8, 9). Layers stack on each other a few Ångstroms apart to create an insoluble black mass (10). Eumelanins are resistant to hydrolysis in acid or base but can be oxidatively decomposed with hydrogen peroxide, especially at alkaline pH (11, 12).

The non-proteinaceous organic jaw residue remaining after exhaustive acid hydrolysis of Glycera jaws represents over one-third of the original dry weight and is morphologically indistinguishable from the whole jaw (3). The substantial acid-resistant residue of Glycera jaws has not previously been chemically examined. The present study more clearly defines the chemical structure of this residue. The results indicate that the residue remaining after acid hydrolysis is definitely a melanin, albeit an unusual one.

Melanin and other polyphenolic networks, often as part of an extracellular matrix, provide many diverse functions in nature (13–15). One of these functions, protein cross-linking, was proposed to have mechanical consequences in insect cuticle (14, 16, 17); changes in the mechanical properties of fungal cell walls have also been correlated with melanin synthesis (18). Melanins resemble other common biopolymers such as \(N\)-acytetyldopa mine-stabilized sclerotins and lignins in being polyaromatic. Unlike the load-bearing sclerotins and lignins, however, the best known melanins are colloidal suspensions associated with the concealment inks of cephalopods and the pigments of integument and neural tissues. To our knowledge, this report is the first clear demonstration that melanin endows a structural biomaterial in a metazoan with mechanical properties that are distinct from those contributed by mineral or structural proteins.

EXPERIMENTAL PROCEDURES

Glycera dibranchiata worms from Maine Bait Company (Newcastle, ME) were dissected for jaws. Pulp tissue surrounding jaws was removed by soaking jaws 24 h in water followed by
the removal of pulp tissue with microforceps. Jaws were dried and weighed.

Hydrolysates—Unless otherwise stated, batches of 4–5 mg of Glycera jaws or Sepia melanin were vacuum-sealed in glass ampoules containing 300 μl of 6 M HCl with 10 μl of phenol and heated at 110 °C. After 48 h, acid and hydrolysate were removed. The solid sample remaining was washed extensively with water and methanol, dried, and rehydrolyzed. Altogether, samples were hydrolyzed 6 days, with the hydrolysis solution replaced after 2 and 4 days. These samples are referred to as hydrolyzed jaws and hydrolyzed Sepia. Glycera jaws were also hydrolyzed in 4 N NaOH at 100 °C, and similar results were obtained.

When applicable, hydrolyzed Glycera jaws and hydrolyzed Sepia melanin were frozen in liquid nitrogen and ground to a powder using a mortar and pestle. These samples are referred to as hydrolyzed jaw powder and hydrolyzed Sepia powder.

General Characterization—After 48, 96, and 144 h of hydrolysis, remaining insoluble jaws were massed, and solubilized hydrolysates at each point were subjected to several assays. Hydrolysates were submitted to ninhydrin-based amino acid analysis using a Beckman Coulter 6300 amino acid analyzer. Hydrolysates were also evaluated using inductively coupled plasma atomic emission spectrometry and furnace atomization atomic absorption spectrophotometry to quantitate metals.

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H₂O₂-based Degradation to Quantify Melanin—Untreated and hydrolyzed Sepia melanin and jaw powder solubilized in alkaline H₂O₂ were analyzed for absorbance at 560 nm to determine total melanin content. Standard curves of untreated and hydrolyzed Sepia melanin were constructed using 0, 0.1, 0.2, 0.5, 0.75, and 1 mg/ml each melanin. Untreated and hydrolyzed Glycera jaw powders were suspended as 1 mg/ml solutions.

One part 10 N NaOH and 2 parts 30% H₂O₂ were added to 37 parts each standard and sample. This mixture was incubated 30 min at 70 °C and centrifuged at 14,000 rpm to remove residual solids, and the visible absorbance of the supernatant was measured. Standard curves of untreated Sepia (R² = 0.9951) and hydrolyzed Sepia (R² = 0.9635) were linear at 560 nm. As hydrolysis may convert some hydroxyindole units to indole quinones, untreated and hydrolyzed Glycera jaw samples were compared with untreated and hydrolyzed Sepia standard curves, respectively.

Solid State CP-MAS NMR—Approximately 40 mg of Glycera jaws were hydrolyzed 72 h at 110 °C in a vacuum-sealed ampoule containing 5 ml of 6 M HCl and 50 μl of phenol. Soluble hydrolysate was removed and replaced with another 5 ml of HCl and 50 μl of phenol at 48 h. After 72 h of hydrolysis, jaw residue was washed extensively and flash-evaporated.

¹³C cross polarization-magic angle spinning (CP-MAS) NMR spectra were measured from whole jaw and hydrolysis residue powdered samples in 4-mm zirconia rotors spinning at 12,000 rpm using a Bruker AVANCE 500 MHz spectrometer. All ¹³C shifts are referenced to tetramethylsilane.

Vibrational Spectroscopy—Hydrolyzed Glycera jaw powder and hydrolyzed Sepia melanin powder were mixed 1:100 w/w with KBr, and the mixtures were ground in a mortar and pestle until uniform color was achieved. Aliquots were pressed into 5-mm pellets and characterized with IR spectroscopy.

IR spectra of pellets were measured in transmission using a Nicolet Magna 850 IR spectrometer over the range of 400–4000 cm⁻¹. Prior to measurement, the sample pellets were dried in a vacuum oven at 105 °C for 15 min. IR analyses were carried out in N₂.

Laser Desorption Ionization Mass Spectrometry—Hydrolyzed Glycera jaw powder and hydrolyzed Sepia melanin powder were analyzed using laser desorption ionization mass spectrometry (19, 20). Hydrolyzed and ground jaw and Sepia samples were dried thoroughly and then packed into small spots on a piece of double-sided tape. No matrix was used in these experiments. An accelerating voltage of 18,000 and 25,000 with a grid voltage of 94 and 93% was used to analyze the Glycera jaw powder and Sepia powder, respectively. Both samples were analyzed in positive ion mode, with 256 scans averaged.

RESULTS

General Characterization—Jaw residue remaining after 48, 96, and 144 h of hydrolysis represents about 50, 42–45, and 38–40% of the total jaw dry weight, at each respective time point (Fig. 1). The mass removed has been almost entirely accounted for as protein, metal ions/mineral, and sulfates. The average composition of the removed protein is Gly- (50%) and His-rich (23%), but viewed from each time point, Gly is more readily released than His. Initially, His:Gly is 0.4 by mole fraction, but by day 4, this ratio jumps to between 3 and 4, and by day 6, it has reached between 7 and 8. The shape of the remaining jaw residue appears very similar to untreated jaws by optical microscopy, although the jaws shrink to ~85% of their original length and width following the complete hydrolysis procedure (Fig. 2).
H₂O₂-based Degradation to Quantitate Melanin—Sepia melanin and jaw residue remaining after hydrolysis resist further degradation by the action of concentrated HCl, NaOH, H₂SO₄, hydrofluoric acid, perchloric acid, alcohols, acetone, and chloroform. Both materials can be solubilized, however, with H₂O₂ treatment, which is accelerated at alkaline pH or elevated temperature. The degradation of melanin by H₂O₂ is known to produce pyrrole acids (11, 12, 21–25), forming an orange-brown chromophore that increases linearly with the amount of melanin degraded (Fig. 3, a and b). This reaction has been previously exploited to detect melanin degradation products purified by high pressure liquid chromatography (12, 21). Here, with Sepia melanin as a standard, we assayed for melanin content based on the absorbance of degradation products at 560 nm.

A standard curve of absorbance at 560 nm following oxidative degradation was constructed using both untreated and HCl-treated Sepia melanin (Fig. 3, a and b). When compared with the appropriate standard curve, untreated Glycera jaws are ~37% melanin, whereas jaw residue following HCl hydrolysis is composed entirely of melanin.

Solid State CP-MAS NMR—Based on expected chemical shifts of model compounds and published NMR assignments (26) of Sepia melanin, the ¹³C CP-MAS NMR data collected for hydrolyzed Glycera jaw residue fit well (Fig. 4). The peaks at ~190 and 170 ppm are assigned as unprotonated and protonated carbonyl groups, respectively. The major features of the spectrum occur between 160 and 100 ppm, which is the range of chemical shifts expected for aromatic and indolic carbons. These peaks are not resolved enough to assign every carbon expected in a melanin monomer unit, but the peaks at 158, 128, and 113 ppm fit well with the expected chemical shifts of a melanin monomer as shown in Fig. 4. Furthermore, aliphatic chemical shifts are also observed in the range of 0–100 ppm, as in published spectra of Sepia melanin. The peaks observed at ~300 and ~50 ppm are attributed to spinning side bands. Altogether, the NMR spectra in Fig. 4 match remarkably well with those of Sepia melanin. The NMR peaks are broad, but this is expected if the linkages between monomer melanin units are relatively heterogeneous.

Vibrational Spectroscopy—IR spectra for hydrolyzed jaw powder and hydrolyzed Sepia are similar (Fig. 5) and fit well with established vibrations in the literature (27–30). A broad band corresponding to phenolic OH stretches is seen at 3400 cm⁻¹, aromatic C–C stretches and COO stretches are visible at 1600–1650 cm⁻¹, and phenolic COH bends and indolic and phenolic NH stretches are visible at 1380–1400 cm⁻¹. In addition, aliphatic stretches and alcohol OH stretches are visible in both samples. These results strongly suggest that in both Sepia and Glycera samples, the residues following hydrolysis are at least partially composed of phenolic rings with carboxyl and alcohol groups. Accepted partial structures of melanin include
indole and pyrrole rings conjugated to form networks with carboxyl and alcohol functional groups.

**Laser Desorption Ionization Mass Spectrometry**—Mass spectrometry of hydrolyzed *Sepia* and *Glycera* powders (Fig. 6) supports that both of these materials are composed of melanin networks. The major peak in the mass spectra of these materials occurs at 362 and 363 for *Glycera* and *Sepia*, respectively. In the mass spectrum of each material, there is a peak at 16, 52, and 189 daltons higher than this major peak. A mass difference of 16 is likely due to an additional hydroxyl group. A dihydroxyindole carboxylic acid would have a mass of 191, so a mass difference of 189 could derive from the corresponding quinone of this subunit (19, 20). Furthermore, the *Sepia* spectrum has a large peak 155 units lower than the major peak at 363, whereas *Glycera* powder has a large peak 182 units lower than the major peak. These can be accounted for by the presence or absence of a pyrrole carboxylic acid (mass 154) and the same group with a carboxyl moiety attached (mass 182) (19, 20). As a highly heterogeneous polymer network, it is not surprising that two functionally different samples of melanin networks would exhibit slightly dissimilar mass spectra.

**DISCUSSION**

Most biological integuments exhibit some degree of melanization, generally between 0.1 and 5% by weight (31–34). In contrast, the melanin in *Glycera* jaw, identified by Raman, IR, UV-visible, and solid state NMR and by laser desorption ionization analysis, represented 37% of the jaw by dry weight. This level exceeds typical melanin contents by an order of magnitude and suggests functions other than pigmentation.

More importantly, by hydrolytically removing all constituents but melanin, *Glycera* jaws did not disintegrate, although melanins are not renowned as cohesive building materials for load-bearing structures. *Sepia* melanin, for example, is a dispersion of aggregated particles, each of which is itself an aggregate of stacked layers of oligomeric melanin units (8, 9, 35). The interactions driving aggregation are thought to be non-covalent (36, 37) because the cohesion between *Sepia* melanin particles is easily disrupted. In contrast, *Glycera* jaw melanin is organized into 200-nm-thick tablets arranged perpendicular to the long axis of the jaw.⁴ We know

⁴ D. N. Moses, J. H. Harreld, G. D. Stucky, and J. H. Waite, unpublished data.
of only one other instance, melanized fungal cell walls (18, 38, 39), in which interactions between constituent melanins suffice to maintain structural integrity after all other molecules have been removed. The mechanism of this long range stabilization of melanins of Glycera jaw is not yet understood but probably involves linkages between melanin particles. This is supported by the fact that the melanin remaining following hydrolytic extraction of protein, mineral, and metal ions retained more than 50% of the initial stiffness of untreated jaws (40). The present results are thus the first demonstration of a melanin network that serves a distinct load-bearing and shape-determining role in a metazoan tissue.

From a chemical perspective, melanins resemble the polyphenolic networks in sclerotized insect cuticles and the lignin matrix between the primary wall and plasmalemma of cell walls (41, 42). In these materials, low molecular weight dihydroxy- or methoxyphenolic precursors undergo complex polymerizations following oxidation. Although polyphenolic networks endow insect cuticles and woody plant tissues with mechanical strength and chemical stability, these effects rely on an intimate association between the networks, cuticular proteins, and chitin or cellulose fibers. Eumelanin has been detected in insect cuticles, but its contribution to mechanical and chemical properties is unknown (42).

Identifying the function of Glycera jaw melanin is an elusive challenge given the multifunctionality of melanins. Reported properties include microbial resistance, semiconductivity, electrical conductivity, light absorption, and metal chelation (14, 15). Although only metal chelation has been observed in Glycera jaw melanin, these properties could explain the apparently exotic choice of framework material of the worm for its jaws. Glycera jaw melanin has been shown to bind copper so tightly that the metal is not extractable by EDTA treatment (40), in contrast to the zinc in clamworm jaws (43) or mineral in teeth and bones (44, 45). It remains to be determined whether copper is chemically bound or phys-
ically entrapped by the molecular constituents of *Glycera* jaws. This metal binding affinity could help organize the constituents of the jaws, namely protein, mineral, and metal, into an effective composite. Indeed, other melanins bind metal ions in vivo (46), and copper ions affect the structure of synthetic melanins (47). Copper binding affinities of synthetic and *Sepia* melanins have been studied (48, 49).

Melanin has been reported in other connective tissues. It is found in the extracellular matrix of the eye and interacts biochemically with constituents such as collagen XVIII (50), but a structural or mechanical role has not been shown. In fact, a recent study showed that the addition of melanin to an assay has no effect on the ability of retinas to contract collagen gels (51).

Melanin is also found in bird feathers and was suggested to be part of the strengthening mechanism of feathers. However, when normalized for the position along the feather, melanized feather barbs have similar breaking stress, breaking strain, and toughness as unmelanized barbs (52).

Fungal cell wall melanin is proposed to cross-link to proteins, thereby lowering cell wall permeability (53) and retaining cell wall toughness as unmelanized barbs (52). Other speculated roles of melanin in fungal cell walls include protection from UV light, hydrolytic enzymes, and host antimicrobials (38, 54).

Based on the present and related studies (40), it appears that *Glycera* melanin provides significant chemical stability for the jaws. The jaws of a related polychaete species, *Nereis virens*, which also contain His-rich proteins and divalent metals but not melanin, provide an interesting comparison. *Nereis* jaws (43, 55) exhibit similar stiffness and hardness properties as the unmineralized portions of *Glycera* jaws; however, they disintegrate after 24 h of hydrolysis. Although the two structures are mechanically comparable, the chemical stability of *Glycera* jaws is much greater. It remains to be determined in what ways the habitats and/or diet of these worms necessitate such differences in chemical stability.

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REFERENCES

1. Michel, C., Fonze-Vignaux, M. T., and Voss-Foucart, M.-F. (1973) *Bull. Biol. Fr. Belg.* 107, 301–321.
2. Voss-Foucart, M.-F., Fonze-Vignaux, M. T., and Jeuniaux, C. (1973) *Biochem. Syst. Ecol.* 1, 119–122.
3. Gibbs, P. E., and Bryan, G. W. (1980) *J. Mar. Biol. Assoc. UK* 60, 205–214.
4. Lichtenegger, H. C., Schoberl, T., Bartl, M. H., Waite, J. H., and Stucky, G. D. (2002) *Science* 298, 389–392.
5. Wegst, U. G. K., and Ashby, M. F. (2004) *Philos. Mag.* 84, 2167–2181.
6. Waite, J. H., Lichtenegger, H. C., Stucky, G. D., and Hansma, P. (2004) *Biochemistry* 43, 7653–7662.
7. Nicolaus, R. A. (1968) in *Chemistry of Natural Products* (Lederer, E., ed) 2nd Ed., pp. 9–12, Hermann, Paris.

8. Cheng, J., Moss, S. C., Eisner, M., and Zschoch, P. (1994) *Pigm. Cell Res.* 7, 255–262.
9. Cheng, J., Moss, S. C., and Eisner, M. (1994) *Pigm. Cell Res.* 7, 263–273.
10. Thatchachari, Y. T. (1976) *Pigm. Cell Res.* 3, 64–68.
11. Ito, S., Wakamatsu, K., and Ozeki, H. (1993) *Anal. Biochem.* 215, 273–277.
12. Napolitano, A., Pezzella, A., Vincenski, M. R., and Prota, G. (1995) *Tetrahedron* 51, 5913–5920.
13. Barr, F. E. (1983) *Med. Hypotheses* 11, 1–39.
14. Wiley, P. A. (1992) *Pigm. Cell Res.* 5, 101–106.
15. Wiley, P. A. (1997) *Int. J. Biochem. Cell Biol.* 29, 1235–1239.
16. Hackman, R. H., and Goldberg, M. (1971) *Anal. Biochem.* 41, 279–285.
17. Sugumaran, M. (1987) *Bioorg. Chem.* 15, 194–211.
18. Money, N. P., Caesar-TonThat, T.-C., Frederick, B., and Henson, J. M. (1998) *Fungal Genet. Biol.* 24, 240–251.
19. Seraglia, R., Traldi, P., Lelli, G., Bertazzo, A., Costa, C., and Allegrini, G. (1993) *Biochim. Biophys. Acta* 1183, 155–161.
20. Napolitano, A., Pezzella, A., d’Ischia, M., Prota, G., and Waite, J. H. (1997) *Tetrahedron* 52, 8775–8780.
21. Ozeki, H., Wakamatsu, K., Ito, S., and Ishiguro, I. (1997) *Anal. Biochem.* 248, 149–157.
22. Pezzella, A., d’Ischia, M., Napolitano, A., Lumbo, A., and Prota, G. (1997) *Tetrahedron* 53, 8281–8286.
23. Wakamatsu, K., Fujikawa, K., Zucca, F. A., Zecca, L., and Ito, S. (2003) *J. Neurochem.* 86, 1015–1023.
24. Adhyaru, B. B., Akhmedov, N. G., Katritzky, A. R., and Bowers, C. R. (2003) *Biochem. Biophys. Acta* 716, 8–15.
25. Biriliska, B. (1996) *Spectrochim. Acta Part A* 52, 1157–1162.
26. Bridelli, M. G., Tampellini, D., and Zecca, L. (1999) *FEBS Lett.* 457, 18–22.
27. Di, J., and Bi, S. (2003) *Spectrochim. Acta Part A* 59, 3075–3083.
28. Watts, K. F., Fairchild, R. G., Starlkin, D. N., Greenberg, D., Packer, S., Atkins, H. L., and Hannon, S. J. (1981) *Cancer Res.* 41, 467–472.
29. Kronstrand, R., Forstberg-Peterson, S., Kagedal, B., Ahnert, J., and Larson, G. (1999) *Clin. Chem.* 45, 1485–1494.
30. Alalug, S., Atkins, D., Barrett, K., Blount, M., Carter, N., and Heath, A. (2002) *Pigm. Cell Res.* 15, 112–118.
31. McGraw, K. J., Safran, R. J., and Wakamatsu, K. (2005) *Funct. Ecol.* 19, 816–821.
32. Clancy, C. M. R., and Simon, J. D. (2006) *Biochemistry* 44, 1521–1529.
33. Bratosin, S. (1973) *J. Investig. Dermatol.* 60, 224–230.
34. Liu, Y., and Simon, J. D. (2005) *Pigm. Cell Res.* 18, 42–48.
35. Eisenman, H. C., Nosanchuk, J. D., Webber, J. B. W., Emerson, R. J., Camesano, T. A., and Casadevall, A. (2005) *Biochemistry* 44, 3683–3693.
36. Money, N. P., and Howard, R. J. (1996) *Fungal Genet. Biol.* 20, 217–227.
37. Moses, D. N., Mattoni, M. A., Slack, N. L., Waite, J. H., and Zok, F. W. (2006) *Acta Biomater.* 2, 521–530.
38. Bernards, M. A., Lopez, M. L., Zajicek, J., and Lewis, N. G. (1995) *J. Biol. Chem.* 270, 7382–7386.
39. Hopkins, T. L., and Kramer, K. J. (1992) *Annu. Rev. Entomol.* 37, 273–302.
40. Broomell, C. C., Mattoni, M. A., Zok, F. W., and Waite, J. H. (2006) *J. Exp. Biol.* 209, 3219–3225.
41. Balooch, M., Wu-Magdi, J.-C., Balazs, A., Lundkvist, A. S., Marshall, S. I., Marshall, G. W., Siekhaus, W. J., and Kinney, J. H. (1998) *J. Biol. Chem.* 273, 539–544.
42. Tai, K., Qi, H. J., and Ortiz, C. (2005) *J. Mater. Sci.: Mater. Med.* 16, 947–959.
43. Sarna, T., Hyde, J. S., and Swartz, H. M. (1976) *Science* 192, 1132–1134.
44. Stepień, K. B., Dworzanski, J. P., Bilinska, B., Porebska-Budny, M., Hollek, A. M., and Wilczok, T. (1989) *Biochim. Biophys. Acta* 997, 49–54.
45. Szpoganicz, B., Gidian, S., Kong, P., and Farmer, P. (2002) *J. Inorg. Bio-
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49. Liu, Y., Hong, L., Kempf, V. R., Wakamatsu, K., Ito, S., and Simon, J. D. (2004) Pigm. Cell Res. 17, 262–269
50. Marneros, A. G., and Olsen, B. R. (2003) Investig. Ophthalmol. Vis. Sci. 44, 2367–2372
51. Smith-Thomas, L. C., Richardson, P. S. R., Rennie, I. G., Palmer, I., Boulton, M., Sheridan, C., and MacNeil, S. (2000) Curr. Eye Res. 21, 518–529
52. Butler, M., and Johnson, A. S. (2004) J. Exp. Biol. 207, 285–293
53. Hutchison, K. A., Green, J. R., Wharton, P. S., and O’Connell, R. J. (2002) Mycol. Res. 106, 729–736
54. Doss, R. P., Deisenhofer, J., Krug von Nidda, H.-A., Soeldner, A. H., and McGuire, R. P. (2003) Phytochemistry 63, 687–691
55. Birkedal, H., Khan, R. K., Slack, N., Broomell, C., Lichtenegger, H. C., Zok, F., Stucky, G. D., and Waite, J. H. (2006) ChemBioChem 7, 1392–1399