AN ABSTRACT OF THE THESIS OF

Makayla SueAnn Fu Ting Gessford for the degree of Honors Baccalaureate of Science in Bioengineering presented on May 29, 2014. Title: Self Assembly of GeO_2 Nanoparticles: A Biomimetic Approach

Abstract Approved:

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Joe E. Baio

Diatoms are single celled eukaryotic microalgae that self-assemble complex SiO_2 nanostructures by sillafin proteins within their cell wall. There has been increasing interest in mimicking the diatom's silica self-assembly process for fabricating metal oxide nanoparticles, namely germanium dioxide (GeO_2). GeO_2 has a higher refractive index than silica, enabling it to be applied in a range of technologies such as fiber optics, optoelectronic devices, and complimentary metal oxide semiconductors. Current GeO_2 nano-fabrication methods require high pressure, temperature, and a great deal of time and resources. Our investigation offers a new approach, one based on the diatom's natural process to manipulate GeO_2. We demonstrated that with this approach, GeO_2 nanoparticles could be fabricated under ambient conditions with a simple protocol. A metal oxide precursor, germanium (IV) ethoxide (GTE), and the protein poly-L-lysine as the biomimetic template (a simplified model of the diatom derived silaffin protein) were placed in artificial seawater (PBS). Three sets of GeO_2 nanoparticles were self-assembled from solutions that contained high (54 mg/mL), medium (28 mg/mL) and low (14 mg/mL) GTE concentrations. Scanning electron microscopy revealed that at high GTE concentrations, only oval shaped GeO_2 nanoparticles (160 nm diam.) were produced while at medium GTE concentrations, cubic (200 nm diam.) and oval (160 nm diam.)

shaped nanoparticles were self-assembled. At low GTE concentrations, indefinite shapes were assembled, described as round agglomerates. Energy dispersive x-ray spectroscopy (EDAX) data at this low GTE concentration reveal elemental nitrogen within the sample, confirming the presence of –NH and/or –N-C bonds and therefore PLL. We hypothesized that the PLL protein acts as a template for the self-assembly of GeO_2 nanoparticles. This has been confirmed by EDAX assay revealing the presence of PLL in nanoparticle samples.

Key Words: Diatoms, germanium, nanoparticles, biomimetic.
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Self-Assembly of GeO_2 Nanoparticles: A Biomimetic Approach

by

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Makayla S. Gessford, Author

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INTRODUCTION

Motivation

There has been an increasing interest in germanium dioxide nanoparticles (GeO_2) because of their blue photoluminescence (Trukhin, 2006) and high refractive index (Nalwa, 2001). GeO_2 has a refractive index of 1.6 to 1.65, compared to that of silica glass (1.45), giving germanium nanoparticles many potential uses in optoelectronic devices (Chiu, 2009). The greater refractivity of germania glass also gives it great potential applications in fiber optics (Patwardhan, 2005) and complimentary metal-oxide semiconductors (CMOS) (Xu, 1996). As a result, researchers have been hunting for novel ways to fabricate germanium nanoparticles. Currently, manipulation of GeO_2 requires the use of bioreactors (Gale, 2011), laser ablation techniques (Riabinina, 2006), or complex reverse micelle systems (Chen, 2007).

Objectives

The primary focus of the work described in this thesis was the fabrication of germanium nanoparticles by a biomimetic process. The goal was to copy the biomineralization process used by diatoms to produce nanoparticles under ambient conditions. The recipe for GeO_2 nanoparticle assembly was adapted from previous work that demonstrates the formation of SiO_2 nanoparticles in solution, but here we were replacing a silicon precursor with a germanium one (Gordillo, 2014). A small homopeptide of L-lysines (PLL) was placed in artificial seawater (buffer) and then allowed to react with the metal precursor in an ambient environment. We hypothesized that the protein ploy-L-lysine would act as a biomimetic template for germanium nanoparticle formation, resulting in self-assembled nanoparticle structures like those observed in nature. During experimentation, the formed nanoparticles precipitated out of solution within minutes. Following nanoparticle assembly, their size, shape, and morphology were assessed by scanning electron microscopy. The fabrication method was then optimized to yield the most uniform germanium nanoparticles possible.

BACKGROUND

The Diatom

Diatoms are single celled eukaryotic microalgae (Kroger, 2007) that can be found throughout the Earth's oceans. Diatoms, along with other unicellular aquatic life, are responsible for the majority of the biomineralization of silica within the earth (Hildebrand, 2008). Biomineralization is the process through which inorganic materials are formed under the control of an organism (i.e. the diatom) (Sumper, 2004). Diatom cell walls are constructed of biosilica; the diatom itself is assembled in a manner such that the top half (epitheca) overlaps the bottom half (hypotheca) (Sumper, 2004). The epitheca and hypotheca enclose the diatom's protoplast; specialized membrane-bound compartments and the site of silica (SiO_2) formation within silica deposition vesicles (SDV, an organelle, see Figure 2) (Kroger, 2008). These SDV's are developed during cell division, and deposit the silica on the surface of the cell via exocytosis of the SDV. Diatoms receive silica in the form of $Si(OH)_4$, orthosilicic acid, prevalent in most water habitats. Through a series of uncharacterized intracellular transports, orthosilicic acid is converted to silica in the form of SiO_2, the form that is found within the SDV's and deposited on the cell walls. Silicon is an essential part of the diatom cell division process because without it, division would cease and the diatom line would die (Kroger, 2008). Biosilica cell walls of various diatom species are shown in Figure 1 (Kroger, 2008).

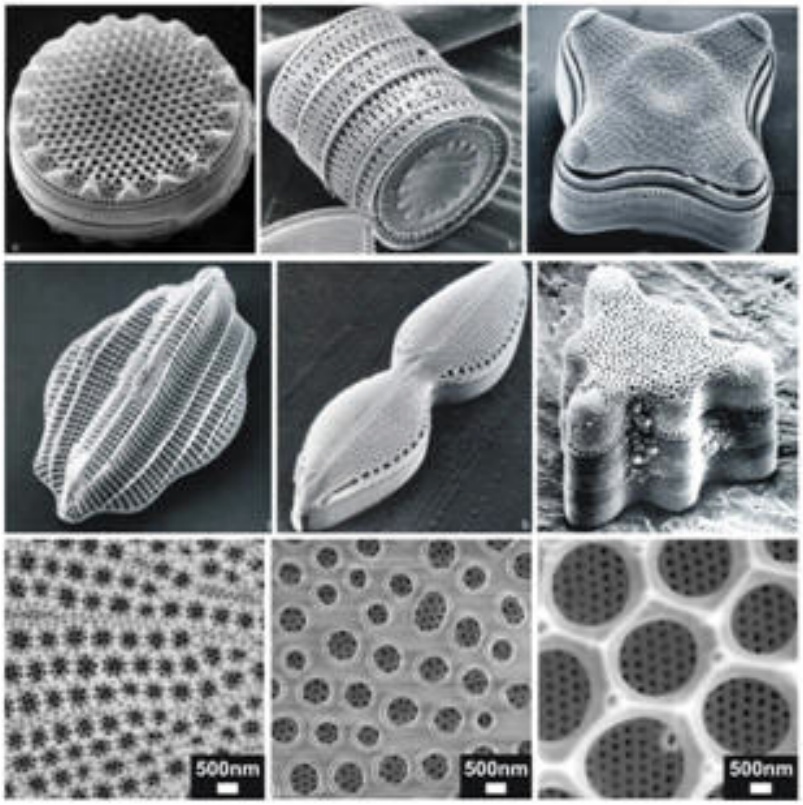


Figure 1. Images of various diatom species highlighting their cell walls. The bottom row gives more detail, displaying the highly ordered deposition of silica at the nano-scale. The bottom row of images shows detail with a 500 nm scale bar.

As shown (Figure 1), diatoms are capable of forming a wide range of perfectly arranged nanostructures. These nanostructures can be patterned to form squares, cylinders, triangles, and other uniquely shaped polygons. The amazing thing about all of these structures is that they are extremely symmetrical and highly ordered on both the micro- and nano- scale. Furthermore, the diatom organism is capable of producing these nanostructures through the help of specific proteins within its cell wall, and without any assistance from the outside world. It is because of their unique capability to create such highly ordered nanostructures autonomously that we are interested in them as a biomimetic model for the self-assembly of GeO_2 nanoparticles.

There are four types unique proteins within a diatom's cell wall: frustrulins, pleuralins, silaffins, and p150's. Frustrulins are general diatom cell wall proteins but are not associated with the cell wall until after silica formation is complete, therefore they do not aid in the formation of biosilica (Kroger, 2008). Pleuralin proteins, which are not present in all diatom species, also do not have a well-defined role in cell wall biomineralization (Kroger, 2008). Instead, they aid diatom cell division serving as the epitheca for one of the sibling cells (Kroger, 2008). The function of the p150 proteins are vague. It is thought that they somehow assist in the biogenesis of the girdle band (overlapping region) by associating with the girdle band after silica deposition (Kroger, 2008). Silaffin proteins are entrapped within the silica cell walls and are a type of phosphoprotein (Kroger, 2008). An overview diagram displaying the diatom cell division process is shown in Figure 2 (Kroger, 2008). This cycle requires both pleuralin and p150 proteins.

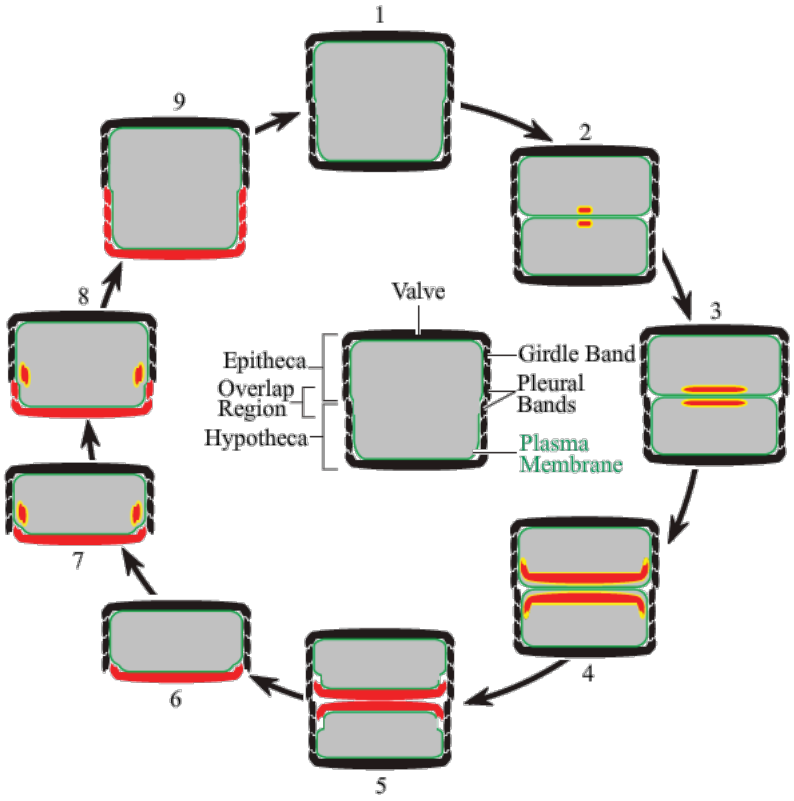


Figure 2. The diatom cell division cycle.

Finally, silaffins are directly embedded within the diatom cell wall and play a role in silica formation (Kroger, 2008). Across the various diatom species, the silaffin proteins carry the same ability to become phosphorylated along with other posttranslational modifications (Kroger, 2008). Phosphorylation, modifications that include attachment of a phosphate or to another group by a phosphate linker, are the structural alterations which allow for the functional properties of the silaffin proteins (Kroger, 2008). Their namesake is also based on this idea; silaffins are proteins with silica affinity (Kroger, 2008).

Recent work has demonstrated that this silaffin-silica biomineralization process can be mimicked in the lab under ambient conditions (Gordillo, 2014). Silaffin protein is placed in a buffer solution under neutral pH and room temperature in the presence of phosphate. Phosphate induces a microscopic phase separation producing polyamine-polyanion loaded microdroplets within the bulk aqueous phase (Kroger, 2008). Once silicic acid is added, solid silica microdroplets solidify within minutes (Kroger, 2008).

Silaffin Model

Diatom derived silaffin proteins are rich with lysine residues (Sasso, 2012). Therefore, the model protein chosen for this investigation is poly-L-lysine (PLL), a short chain homopolymer of L-lysine amino acids. This peptide was chosen because of its abundance of reactive primary amines and simple structure (Figure 3). Because silaffin proteins contain many lysine amino acids, it was deemed essential to evaluate the function and importance of their role in nanoparticle formation by selecting a peptide comprised of only lysine residues.

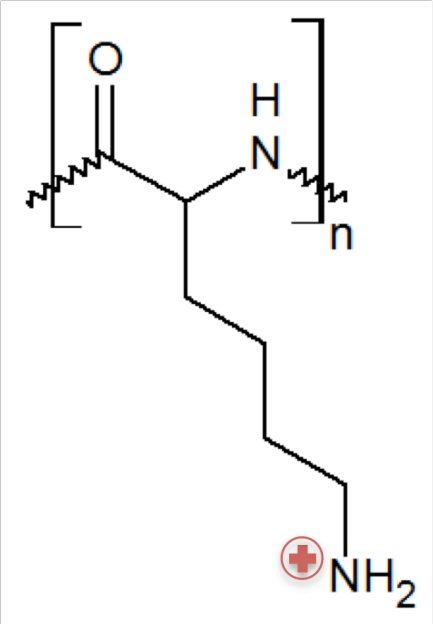
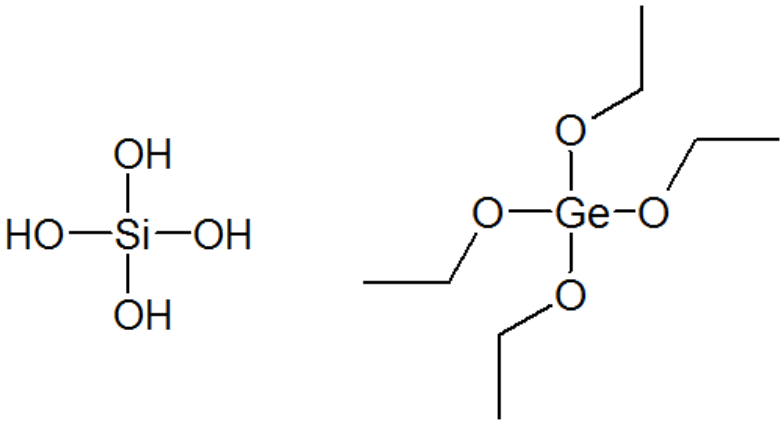


Figure 3. General structure of poly-L-lysine at pH 7.4 where n=3-14.

The PLL protein must have something to assemble with, which is where the choice of a metal precursor is essential. Germanium (IV) ethoxide (GTE) was chosen because of its similarities to orthosilicic acid, the precursor to SiO_2 formation within the diatom

(Kroger, 2008). A comparison of the two precursors, orthosilicic acid versus germanium (IV) ethoxide is illustrated in Figure 4.



(a) (b)

Figure 4. (a) Orthosilicic acid, as found in most water habitats and taken up by diatoms. (b) Germanium (IV) ethoxide, the germanium precursor chosen for the experimental procedures in this thesis because of its similarities to orthosilicic acid.

The chemical structures are almost identical with the exception of the terminal ethyl groups on GTE as opposed to alcohol groups on the orthosilic acid. Because of their similarities we believe that GTE will also form nanoparticles by mimicking the interaction between the free amines within the peptides and orthosilicic acid – the interaction that drives biomineralization in diatoms (Figure 5).

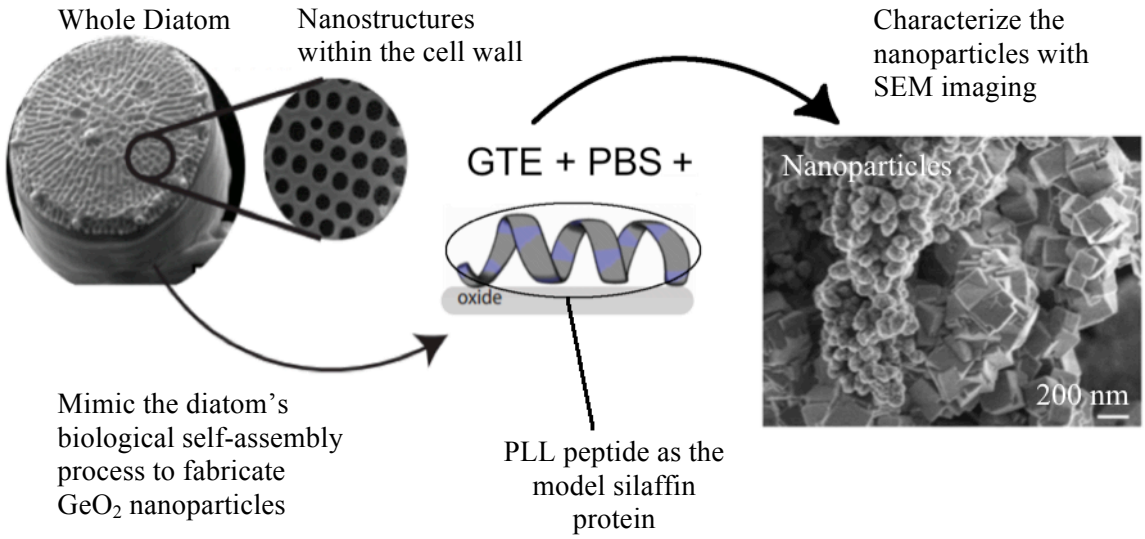


Figure 5. Flow diagram of the overall work presented in this thesis highlighting the role of the PLL protein in nanoparticle formation.

MATERIALS & METHODS

The metal precursor germanium (IV) ethoxide (GTE) and macromolecule poly-L-lysine (PLL) were both purchased from Sigma Aldrich. PLL was used as a simplified model of the larger, more complex silaffin protein. Again, we believe that PLL will provide the same basic function as the sillafin protein - act as a template for nanoparticle self-assembly. Experiments were completed in the presence of phosphate buffered saline (PBS), pH 7.4 at 25 °C: 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride.

Preparation

GeO_2 nanoparticles were assembled from a PBS-protein-precursor solution. Three sets of GeO_2 nanoparticles were self-assembled from solutions that contained a low (14 mg/mL), medium (28 mg/mL), or high (54 mg/mL) GTE concentration. The GTE was added to a 2 mg/mL PLL buffer solution and immediately after adding the germanium precursor, the solution became cloudy and a noticeable white precipitate fell out of solution. The PLL-GTE in PBS mix was then vortexed for 5 minutes to allow for complete mixing, after which it was allowed to sit for 20 minutes. A series of centrifugation cycles followed: samples were spun at 1400 rpm for 5 minutes at 30 °C, the supernatant was discarded, and the precipitate was rinsed with 200 μL of ultrapure water. The rinse – centrifugation step was repeated twice and the accumulated solid was allowed to dry overnight in a sterile dessicator. Figure 6 shows a flow diagram of the general experimental procedures described here. Control experiments were completed synonymously in which no PLL

was initially added to the PBS buffer. These control experiments contained the GTE precursor and PBS buffer only.

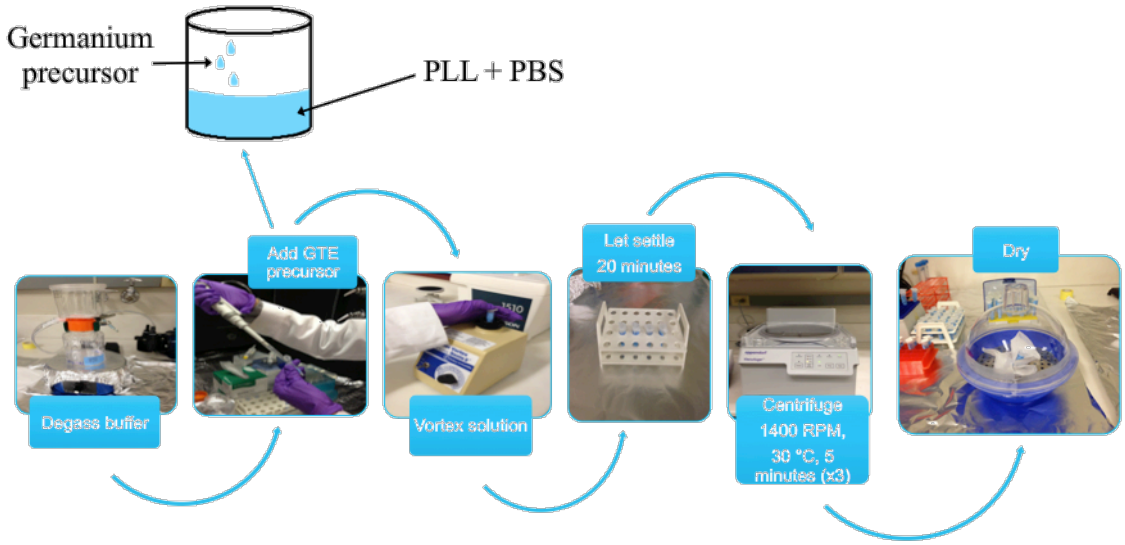


Figure 6. A flow diagram depicting the general experimental procedures.

Characterization

Samples were sent to the Max Planck Institute for Polymer Research in Germany for characterization. The morphology, microstructure, and composition of the samples was investigated by scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDAX) on a LEO 1530 instrument. SEM produces an image by focusing a beam of electrons under vacuum that raster across the sample surface, and detecting the secondary electrons and backscattered electrons that sputter off as a result. These detected electrons ejected from the sample are converted into an image, which can be analyzed. SEM images yield information about the morphology of the structures, as well as details about the size and surface topography. Thereby providing a straightforward assessment of the success or failure of the self-assembly protocol and direct comparison

to literature. EDAX focuses a beam of electrons under vacuum onto the sample, causing x-rays to eject from the targeted area. These x-rays are indicative of the atom from which they came from, allowing for both a quantitative and qualitative elemental assessment of the sample. This would provide direct information about the presence or absence of PLL through the indication of elemental nitrogen (proteins contain amide bonds composed of nitrogen).

RESULTS

The overall goal of this investigation was to test the hypothesis that PLL in solution with the metal precursor germanium (IV) ethoxide self-assembles monodispersed nanoparticles. We believed that self-assembly would not occur without the PLL and that the GTE-buffer solution should not precipitate at all, or if it did, it should produce highly dispersed (non-uniform in size and shape) particles. To test this hypothesis, GeO_2 nanoparticles were self-assembled from buffer solutions that contain only the metal precursor and a combination of PLL and precursor. Following particle assembly, SEM images were collected form the dried product formed to provide a qualitative assessment of particle size, shape, and distribution.

A SEM image taken from particles produced from the low GTE concentration experiment can be found in Figure 7.

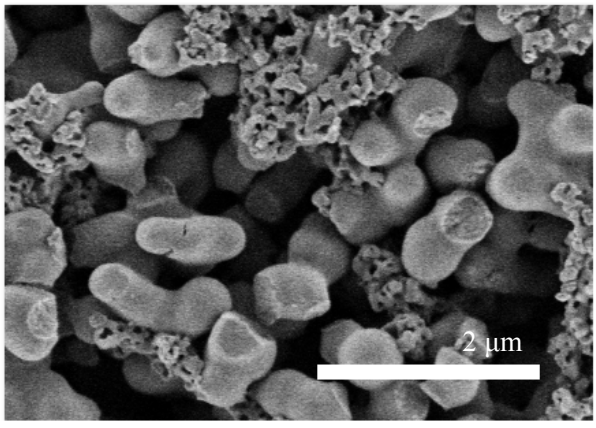


Figure 7. SEM image of experiments conducted with GTE at 14 mg/mL in PBS buffer with PLL. The scale bar is 2 µm.

The control sample (no PLL in solution) at this low concentration of GTE did not yield any product. The assembled particles shown in Figure 7 are fused spheres of

approximately 480 ± 100 nm diameter. Nanoparticle diameters are reported as the arithmetic mean of five randomly chosen particles - the sizes of which were determined with a ruler and the scale bar. EDAX analysis of this sample reveals germanium, sodium, oxygen, carbon, and nitrogen were all present within the sample (Figure 8). The germanium precursor, GTE contains germanium, oxygen and carbon, thus those elements were all expected. Sodium was present as a residue from the PBS buffer, and is not significant. The nitrogen, as indicated by an intense peak at approximately 0.04 keV, is indicative of the PLL peptide, which contains both –NH and N-C bonds.

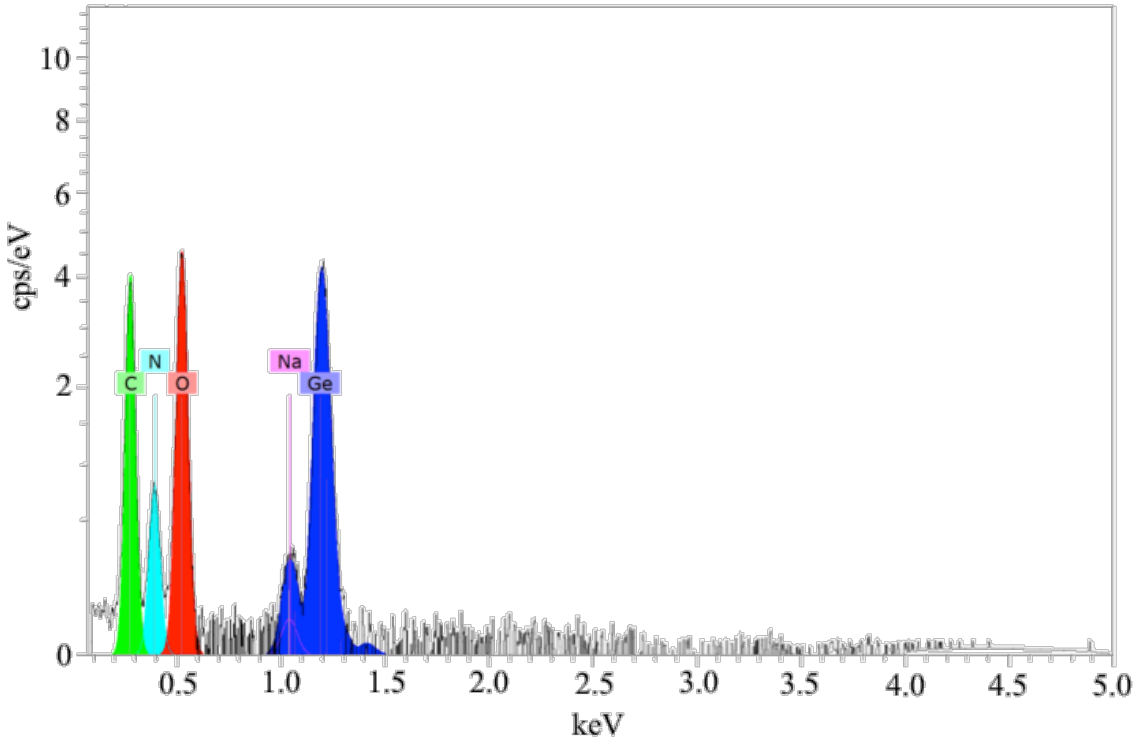
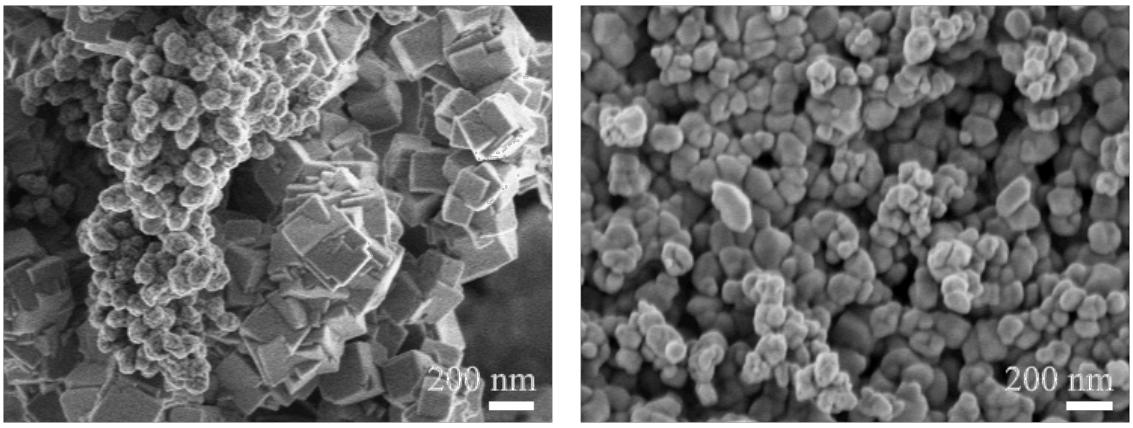


Figure 8. EDAX data of the low concentration GTE product. The nitrogen peak indicates the presence of protein within the sample. The slight amount of sodium is residue from the buffer.

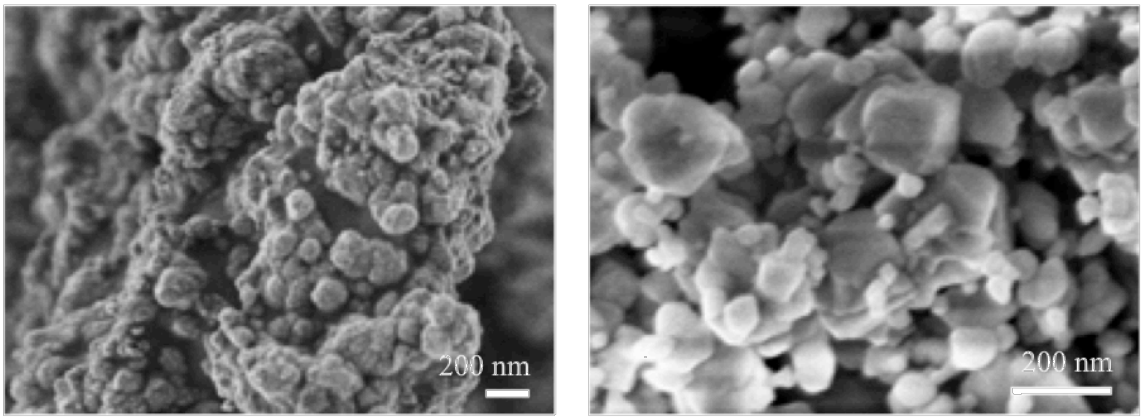
SEM images of the product obtained from experiments with medium concentrations of GTE are shown in Figure 9.



(a) (b)

Figure 9. SEM images of experiments conducted with GTE at low concentration in PBS buffer (a) with PLL and (b) in the absence of PLL (control).

Here, the addition of PLL appeared to drive the formation of cubic nanoparticles. The average length of the cubic nanoparticles is 220 ± 100 nm, while the oval shapes seen in conjunction with the cubes have an average size of 120 ± 54 nm. Product formed in the absence of PLL does not show any distinct shapes, and the polygon formations are highly dispersed in size. The control experiment under these conditions did not always yield a solid product. More often than not, as in the case when a low concentration of GTE was used, if no PLL protein was added to the solution, nothing would precipitate out of the solution. For this case, a solid product would occasionally form, warranting the direct comparison by SEM of what the formed particles look like for qualitative analysis. This is also the case for formed nanoparticles when a high concentration GTE was used (Figure 10). Generally, nothing would precipitate out of solution for the control experiments, however, this phenomenon would occasionally occur. The SEM images of the formed products using a high concentration of GTE show the results of experiments conducted in the presence and absence of PLL.



(a) (b)

Figure 10. SEM images of experiments conducted with high concentrations of GTE in PBS buffer (a) with PLL and (b) in the absence of PLL macromolecule (control).

When germanium was added to solutions of high GTE concentrations (50 mg/mL and greater), no cubic formations were noticed. The formed particles have no distinct shape and appear to be one large agglomerated mass of round structures. The product formed in the absence of PLL (Figure 10 (b)) does not have any distinct individual shapes, and the polygon structures present are very disperse in size. This is exactly similar to the formed product in the control experiment when a medium concentration of GTE was used (Figure 9 (b)).

In product formed using a medium concentration of GTE, cubic formations were observed (Figure 9 (a)), whereas product formed using a high concentration of GTE did not show any cubic structures. Results from the low concentration of GTE are less conclusive about the size and morphology of the particles due to outstanding experimental conditions that could have negatively affected the outcome (e.g. aging precursor, contaminated peptide stock solution, etc). Combined, these experiments show that, given the stock concentration of PLL in solution (2 mg/mL), a very specific, and

relatively medium concentration of germanium precursor is needed to form cubic nanoparticles. The addition of too much germanium is overwhelming, and the PLL is able to assemble with only so much, yielding the double shape seen in Figure 9 (a). We speculate that the cubes are the result of GTE assembling with PLL, and that the oval shapes are the result of excess germanium precipitating on its own as it would without the presence of PLL. Figure 11 shows a simple diagram describing the routes to germanium formation to either nanoparticles (N), or crystals (C).

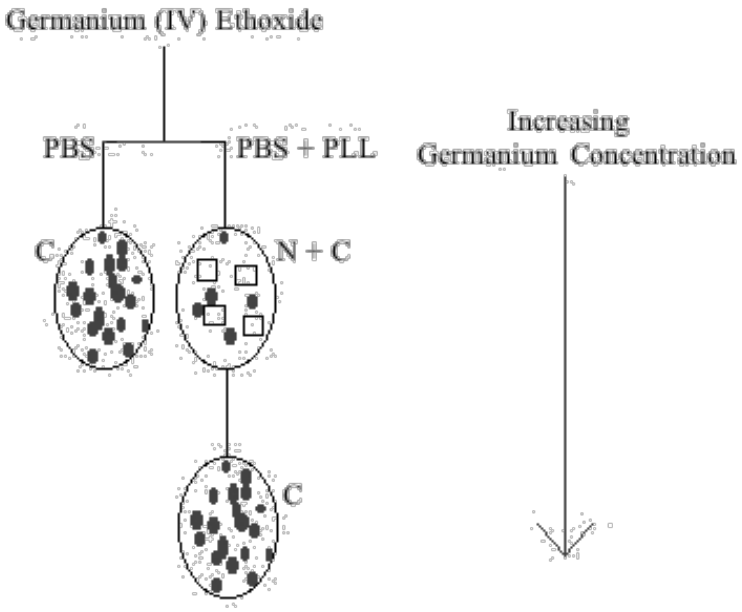


Figure 11. Routes to germanium precipitation of nanoparticles (N), or crystals (C). Germanium (IV) ethoxide concentration increases from top to bottom.

DISCUSSION

A comparative study done by Davis *et al*. explored the use of PLL as a template for GeO_2 nanoparticle formation from the same GTE precursor used in this thesis (Davis, 2007). Their work revealed that germanium (IV) ethoxide rapidly hydrolyzes in pure water, giving rise to soluble species, most of which are deprotonated germanic acid, following the proposed series of reactions (Davis, 2007):

$$Ge(OC_2H_5)_4 + 4H_2O \rightarrow Ge(OH)_4 + 4C_2H_5OH \quad (1)$$

$$Ge(OH)_4 + OH^- \rightarrow Ge(OH)_3O^- + H_2O \quad (2)$$

$$Ge(OH)_3O^- + OH^- \rightarrow GeO_2(OH)_2^{2-} + H_2O \quad (3)$$

The dissociation of water is accounted for in the following reaction, Equation (4), with a pK_w of 14 at 25 °C.

$$H_2O \xleftrightarrow{K_w} OH^- + H^+ \quad (4)$$

This study took advantage of similar experimental conditions; GTE was chosen as the metal precursor, poly-L-lysine as the protein backbone for nanoparticle formation, and all tests were conducted under mild conditions (e.g. room temperature, gentle agitation). In this previous work, when a GTE concentration of ~46 mg/mL was used, just slightly under the high concentration GTE experiment of this study, large, cubic, and polyhedral nanoparticles were observed of approximately 500 nm diameter. Davis *et al.* also

observed that nanoparticle size decreased with increasing GTE concentration and increasing amounts of hexagonal-phase germania crystals coexisted with the nanoparticles at relatively very high GTE concentrations. In their study, increasing GTE concentration to ~58 mg/mL, 6 mg/mL more than the high concentration GTE study presented here, smaller and distinctly more cubic nanoparticles of approximately 250 nm diameter with increased uniformity in size and shape were observed. The authors Davis *et al.* concluded that germania crystals form when high concentrations of GTE are used, synonymous to the results found in this study (Davis, 2007). They observed that germanium nanoparticles and crystals could coexist, exactly what is hypothesized to be happening in Figure 9 (a).

Patwardhan *et al.* in their study utilized similar methods to prepare germanium nanoparticles (Patwardhan, 2005). The macromolecule poly(allylamine hydrochloride) was used in the presence of postassium phosphate buffer (pH 7), and GTE was added to solution at concentrations ranging from 22.4 – 54.3 mg/mL. This GTE range is right at the middle of those concentrations presented here and at the highest is identical to our high concentration experiment. The addition of GTE to solution yielded spherical particles, ranging from 0.4 to 3 μm in diameter, estimated by SEM imaging. Authors did not observe well-defined precipitate in the absence of macromolecule, contrary to observations in this study. X-ray diffraction assays of samples determined the absence of crystallinity (concluded by lack of peaks in data), and it was deemed the product formed amorphous germania particles.

A similar approach by Sewell *et al*. investigated the use of PAMAM dendrimer solutions ranging from 35.5 mg/mL to 1421 mg/mL in 200 μL of phosphate buffer (100 mM, pH 7.5) or DI water for the biomimetic formation of GeO_2 nanoparticles (Sewell, 2008). PAMAM dendrimers are large, repetitively branched amine molecules, compared to PLL, which is a long-chain smaller homopolymer of L-lysine. Their approach utilized the same germanium precursor, GTE, at a constant concentration of 27.8 mg/mL in all experiments, exactly the same used in the medium concentration tests of this study.

The dendrimers used were PAMAM generations 0,2,4,6, all terminated by an amine. Generation 4 (G4) when used as the template for GeO_2 formation with GTE as the metal precursor produced nanoparticles of 80±60 nm diameter in water and 350±150 nm in phosphate buffer (Sewell, 2008). Nanoparticles formed in water were amorphous in shape and few were distinctly spherical. Those formed in phosphate buffer produced more spherical nanoparticles of greater uniformity and consistency. Sewell *et al.* believe that particles formed in phosphate buffer have a consistently larger diameter due to the presence of cations in solution. Thereby neutralizing the surface charge of the nanoparticles as they form, allowing them to stay in solution longer producing larger particles (Sewell, 2008). With all PAMAM generations, this pattern was observed; larger diameter nanoparticles were formed in phosphate buffer solution opposed to DI water. Control solutions, those that contained phosphate or DI water as the solvent and no PAMAM dendrimer did not form nanoparticles after the addition of GTE.

The results found by Sewell differ from those found in this report greatly, as no nanoparticle or crystal formation was observed at all in the absence of a template for self-assembly (i.e. PAMAM). There are several factors that could explain the solid germanium precipitate seen in some of the control experiments within this thesis. The GTE germanium precursor can easily become oxidated if it comes in contact with the air. It is possible that many times in our experiments the GTE reacted with the air before reaching the PLL and PBS solution, meaning that the product seen would be the result of the GTE oxidizing before reaching the buffer solution. Additionally, aging precursor as well as poorly degassed buffer or other human error could have come into play. If all reagents are not fresh and the procedures completed with great care, the resulting product may be contaminated.

Hypothesis of Germania Formation

PLL was chosen as the backbone for self-assembly of germanium nanoparticles. PLL is a homopolymer of L-lysine amino acids with a slight positive charge given by the side chains of the lysine amino acids. When placed in a negatively charged environment, PLL increases electrostatic interactions, such as in the presence of a cell membrane (Sitterley, 2008). Strong electrostatic adsorption to metal surfaces is also expected of PLL, as demonstrated by Roth and his colleagues in their study of van der waals and electrostatic forces' contributions to protein adsorption to surfaces (Roth, 1993). The addition of GTE to the solution comprised of PBS and PLL initiates the immediate hydrolysis of GTE to germanic acid [$Ge(OH)_4$] (the analog to the precursor seen in diatoms, orthosilicic acid) which is further deprotonated as previously discussed by Davis *et al* (Davis, 2007).

The deprotonated germanic acid then assembles with positively charged poly-L-lysine to form the cubic structured nanoparticles (Figure 9 [a]). A separate side reaction is hypothesized to occur; excess germanic acid condenses to form solid form germania crystals as proposed by the reaction in Equation (5) (Rimer, 2007). This is theorized to form the oval germanium crystal structures seen in Figure 9 (a).

$$Ge(OH)_{4,aq} \rightarrow GeO_{2,s} + 2H_2O \quad (5)$$

This reaction scheme requires a Gibbs free energy of ΔG_c= -7.8 kJ/mol for the overall condensation of aqueous germanic acid to a bulk solid (Rimer, 2007).

An alternate route to the germania crystals is that of rapid nucleation of the free GTE precursor. Nucleation is the initial process of crystallization, and is the action of forming a nucleus, which must overcome an initial energy barrier (Richard, 2006). This was also hypothezised to occur in the germania crystallization observed by Davis *et al.*, 2007.

Recommendations for Future Research

Continued work includes additional assays on existing stored nanoparticle powders to develop a more complete analysis of the formed product. Infrared spectroscopy (IR) will give a chemical breakdown of the samples, to determine if PLL is present in the remaining precipitate at all, or if the structures seen are merely germania crystals. IR will additionally give information about the structure of the PLL interacting with the GeO_2, if

it is a β-sheeet or α-helix. Additionally, x-ray diffraction could help determine the type of germania structures formed; amorphous versus crystalline or rutile.

The hypothesis - that only cubic germanium nanoparticles should form when low GTE concentrations are used holding all other parameters constant, still needs to be fully tested. This is assuming that further assays would show that the medium concentration GTE samples confirm the presence of PLL whereas the high concentration GTE samples should contain almost none. Once additional IR assays are completed, conclusions can be made about the PLL interactions with the GeO_2, and further investigations to forming other germanium oxide nanostructures can be done. Knowing how this interaction works given the formula presented in this thesis, small experimental alterations can be made to pursue the formation of germanium nanorods, nanospheres, and other self-assembled nanostructures.

Conclusions

The work presented here describes a possible recipe for GeO_2 nanoparticle formation utilizing the peptide PLL under ambient conditions. The peptide has shown to act as the template for nanoparticle growth, however, only when exposed to specific concentrations of GTE. If a relatively high concentration of GTE is used (above 50 mg/mL) the PLL will not be able assemble with the precursor, and the excess germanium will crystallize on its own. At relatively medium concentrations of GTE (27.8 mg/mL) the PLL assembles with as much GTE precursor possible, yielding cubic germanium nanoparticles while at the excess GTE precursor precipitates to crystals. Optimal experimental

conditions for GeO_2 nanoparticle fabrication using the PLL peptide under ambient conditions should be strived for in future studies. Further IR assays will yield information about the interactions between the PLL peptide and the germanium oxide within the formed nanoparticles. Once this is fully characterized, the recipe for nanoparticle self-assembly may be altered to strive for the formation of other various germania nanostructures.

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