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Fluorescent Pressure Response of Protein-Nanocluster Polymer Composites

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14. ABSTRACT

This research focuses on the uses of polymer gold nanocluster (PNC) composites as pressure sensitive indicators of brain damage. The PNC composites are made up of protein coated gold nanoclusters and a styrene-ethylene/butylene-styrene (SEBS) mineral oil composite, invented at the US Army Research Laboratory. The PNC composites were mechanically tested using a custom made setup and an Instron frame under various pressures that match different levels of damage. The stress levels studied in this technical note are lower than those previously studied by other groups and provide a unique perspective. The data shows that fluorescence quenching is present throughout the PNC samples and can be linked to mineral oil content. This research lays the groundwork for future pressure studies and identifies a key issue when using oil inside these PNCs.

15. SUBJECT TERMS

protein stabilized nanoclusters, pressure response, pressure cell, fluorescence, polymer matrices

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Contents

List of Figures

Acknowledgments

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1.   Introduction and Background

Gold nanoclusters, specifically protein stabilized gold nanoclusters, have been heavily researched and have shown a myriad of possible applications including uses in sensors,[1,2] bioassays,[1,3] and imaging.[4,5] Gold nanoclusters are sub 2 nm nanoparticles and do not exhibit plasmon resonance like nanoparticles.[6] Instead, gold nanoclusters exhibit fluorescent effects similarly to semiconductor-based quantum dots and are not nearly as toxic due to their lack of heavy metals.[7] This makes them a prime candidate for biological applications and *in-vivo* sensing, imaging, and labeling.[5,8] Due to their role as fluorophores and desirable biocompatibility, nanoclusters can take over many possible applications from more toxic fluorophores. These applications include mapping protein interactions or monitoring drug distribution in a system.[2,9]

There are several synthesis routes to create nanoclusters; however, we focused on the protein stabilized synthesis method[10] as the protein shell confers interesting mechanical sensing properties as observed in our previously published work and work by Zhang et al.[11,12,13] In this method, the protein will stabilize and reduce the gold nanoclusters while making them more suitable for *in vivo* uses.[2,6] Spurred by the research and novel findings of Zhang et al. and previous work from our group, which showed a positive linear relationship between fluorescence and pressure; we sought to design a composite system that would take advantage of this relationship and exploit it to model brain damage. Zhang et al. hypothesized that the pressure dependence of the bovine serum albumin:gold nanoclusters (BSA:AuNCs) was due to protein ligands compressing around the gold nanoclusters. We tried to use a setup that retains the same working principle as Zhang et al. while allowing us to use solid samples and create a model for brain damage. To this end, we used styrene-ethylene/butylene-styrene (SEBS) as the matrix to simulate brain tissue and examined the emission of BSA. The SEBS system is a system engineered at the US Army Research Laboratory (ARL) by Dr Randy Mrozek and colleagues, which was designed to specifically mimic the modulus of brain tissue.[14,15]

Brain injury, specifically shock-induced brain injury, has been an area of great interest. With recent controversies around concussions, this research is becoming more important. Previous brain injury models have used a hard outer shell and soft gel[13] to model brain damage from pressure waves. Our eventual aim is to begin to understand brain trauma through the use of synthetic models utilizing brain-mimicking polymer composites doped with pressure responsive protein nanoclusters at traumatic brain injury-relevant pressures. Recent research by Alley et al. shows that their brain model feels an amplification of the force from

shockwaves (i.e., shockwaves in the range of 15–108 psig [0.1–0.74 MPa] actually feel like an impact in the range of 300–500 psig [2.07–3.44 MPa] on the brain). Generally, pressures above 200 psig (1.38 MPa) cause fatality and 29–75 psig (0.2–0.52 MPa) cause lung damage.[16] In accordance with this data, the range of 0–6 MPa is extremely important for understanding concussions and brain damage and has not been properly explored. Although, Zhang et al. investigated a wide range of pressures they focused more on high pressures (up to 2 GPa) and only investigated BSA:AuNC in an aqueous solution.[11] More recently, reports by West et al. show a similar upward trend in fluorescence in pressures as low as 50 MPa; however, this is still an order of magnitude above the targeted pressure range to study brain damage.[12,13]

The purpose of this report is to use BSA:AuNCs in a matrix that closely resembles living tissue and to characterize the pressure response of the clusters in this matrix. Our data indicates that there is a positive pressure dependence for BSA:AuNCs in 15% SEBS/85% mineral oil up to 2 MPa, which is consistent with previous unpublished data from our group using composites of the same nature. After this pressure regime the dependence is lost and a decrease in observed fluorescence can be detected, which can be attributed to loss of observed pressure due to an inefficient design in the pressure vessel or a quenching interaction between the mineral oil in the composite and the BSA:AuNCs. Interestingly, no pressure response can be observed with the 5% SEBS/95% mineral oil. We predict that the mineral oil is indeed quenching the fluorescence of the clusters from the data presented in this technical note.

2. Synthesis of Materials

2.1 Synthesis of BSA-Stabilized Gold Nanoclusters

To synthesize the BSA:AuNCs, HAuCl4 (5 mL, 10 mM) was mixed with BSA (5 mL, 50 mg/mL) at 37 ℃ and stirred rapidly. The mixture was brought up to a pH of approximately 12 through the addition of 0.5 mL 1 M NaOH. The solution was left to incubate at 37 °C for 12 h after which it gained a brown tint. When illuminated by ultraviolet (UV) the BSA:AuNC glowed red. Centrifugation was used to remove any impurities and the BSA:AuNC solution was lyophilized for later use.

To manufacture the BSA:AuNC and control quantum dot (QD) composites, BSA:AuNCs and QDs were dispersed into SEBS at a temperature of 85 °C. BSA solution (250 µL of 25 mg/ml BSA:AuNCs into 10 mL of SEBS matrix) and QD (4 µL into 10 mL of SEBS) were doped in various SEBS concentrations

(5% and 15%). The composite was left to cool and solidify. Before the test began the BSA:AuNC and QD composites were heated to 70 °C to allow transfer into the load cell. The cell was loaded in a sandwich configuration where 15% SEBS (85% mineral oil 15% SEBS, heated to 120 °C) was drop cast into an acrylic load cell, then the BSA:AuNC/SEBS or QD/SEBS composite was poured on top, after which a final layer of 15% SEBS was dropped to protect the composite. Each layer was allowed to cool 5–10 min before the next layer was added to prevent diffusion of the BSA:AuNC or QD/SEBS into the top and bottom. This sandwich-based pressure setup protected the doped composites from direct contact with the mandrel and ensured that there was no loss of doped composite from the load cell due to leakage.

2.2 Pressure Generation Setup

To apply pressure to the BSA:AuNCs, an acrylic load cell was employed for hydrostatic pressure testing (Fig. 1).
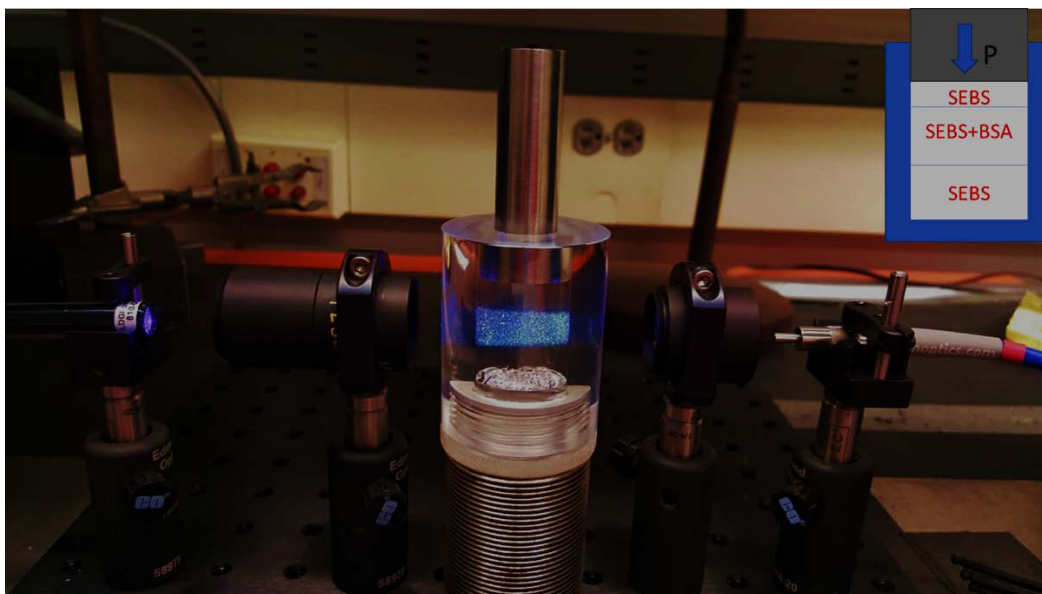

Fig. 1   Instron testing setup for fluorescence change. UV light travels through optics and goes to a detector, which can identify the changes in the emission of BSA. The acrylic load cell sits in the middle of the path between the UV source and detector. In the upper-right-hand corner you can see the composition inside the acrylic cell.

The pressure testing was carried out using an Instron frame. As previously stated, the acrylic load cell used a sandwich structure to protect the BSA:AuNC/SEBS composite from direct contact with the mandrel. The sandwich structure can be seen in the upper right inset of Fig. 1. The top and bottom are composed of 15% SEBS and the middle layer has the BSA:AuNC SEBS composite that needed to be tested. The composite was tested up to 400 lb$_f$ (6.24 MPa) in 50 lb$_f$ (0.78 MPa) increments.

The fluorescence was measured using a detector and a UV source was used to excite the BSA at approximately 370 nm. The pressure setup itself used a UV light source, which was collimated to ensure wide field excitation of the gel and passed through an OD4 550-nm filter to remove any infrared (IR) emission from the UV source, then through the sample and finally passed through an OD4 575-nm filter to block the UV and hit the detector.

2.3 Emission Measurements

The measurements were done using a Jaz detector system and an approximately 370-nm light source for excitation of the clusters. The measurements were done with an integration time of 500 ms with 3 averaged scans for each 50 lb_f (0.78 MPa) increment. Each experiment was repeated 5 times for consistency.

2.4 Data Analysis

The runs were averaged out and plotted so that each load was represented by one spectra (as seen in Fig. 2). The maximum emission of each run was taken and plotted to create a trend line representative of the behavior of the composite.
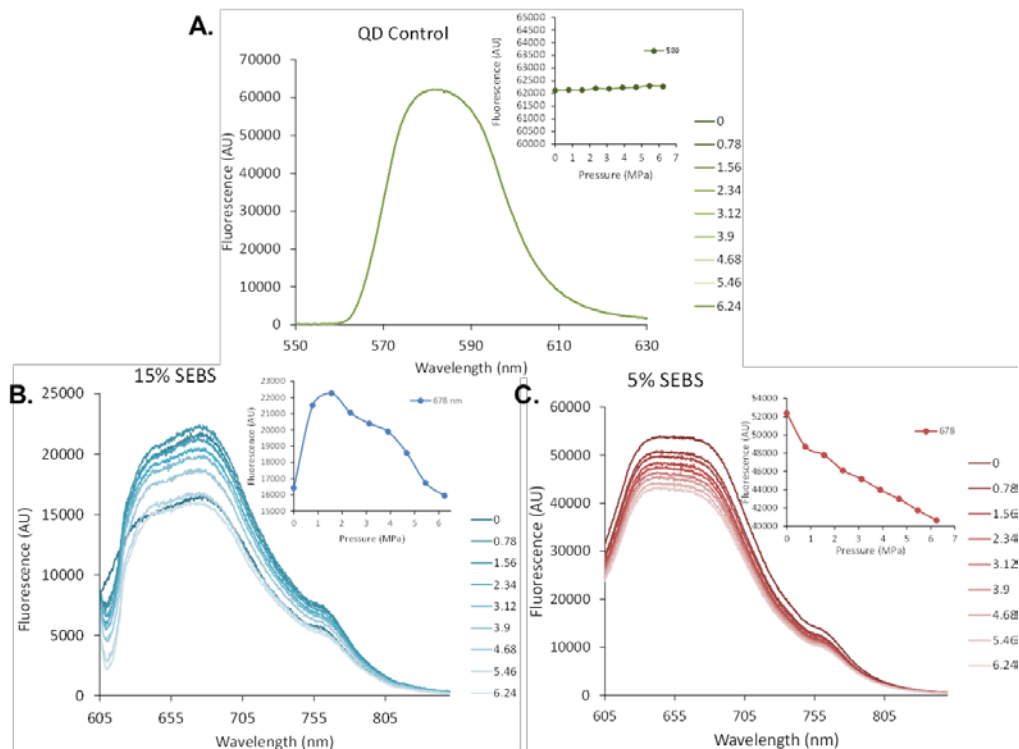

Fig. 2   This figure shows the emission data of the SEBS composites. a) QD control in 15% SEBS, b) BSA:AuNC in 15% SEBS, and c) BSA:AuNC in 5% SEBS. Each line represents an averaged 0.78 MPa interval.

3.   Results and Discussion

A baseline measurement of the emission spectrum of the BSA:AuNCs in aqueous solution and while embedded in the SEBS matrix was taken to observe any matrix effects on the emission profile (Fig. 3). The emission spectra shows peaks for aqueous BSA:AuNCs in the 670–680 nm range, which shifts to approximately 640 nm upon incorporation into the SEBS matrix. These spectra are consistent with the fluorescence spectra of BSA:AuNCs found in literature.[10] Notably, the BSA:AuNCs experience peak broadening and blue shifting upon incorporation into the polymer matrix. This observation is indicative of an interaction between the protein and the SEBS matrix. For example, the peak broadening may be an effect of an increased bond length between the nanocluster core and the surrounding protein ligands.
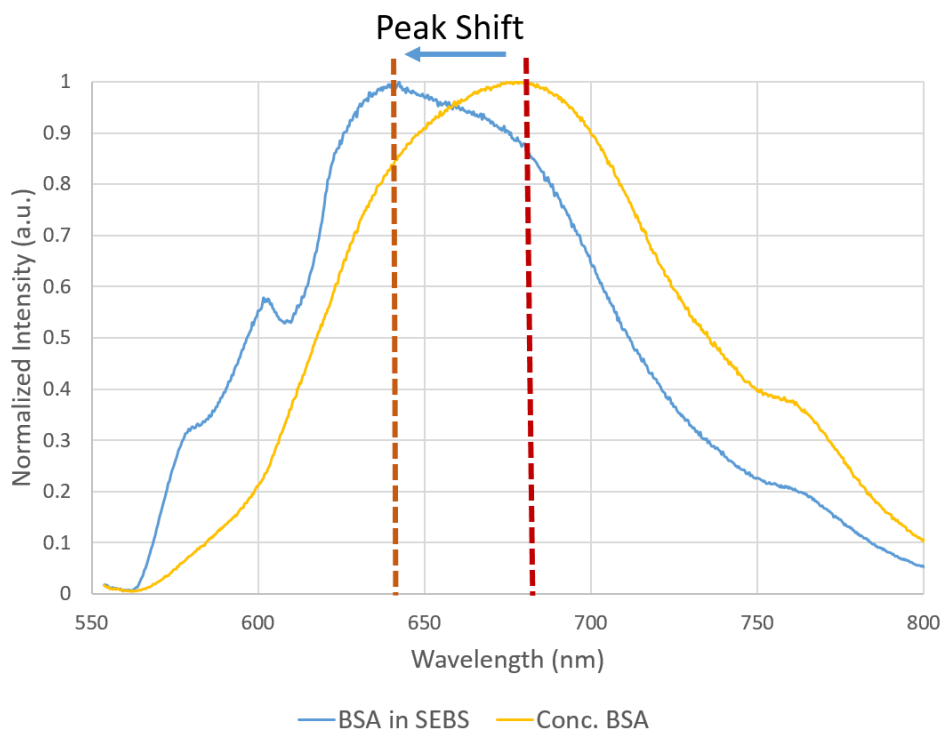
Fig. 3   The emission spectra of BSA and BSA in SEBS upon excitation at 370 nm

Instron mechanical testing revealed an unexpected trend when comparing both BSA:AuNC-SEBS matrix concentrations (Fig. 2). The BSA:AuNC-15% SEBS shows an initial positive response of up to 2 MPa; however, after this point there is a loss of pressure dependence paired with a decrease in fluorescence. The initial positive response matches previous unpublished work generated by our group using BSA:AuNCs in 15% SEBS without the sandwich structure (Fig. 4).

Fig. 4 **Positive pressure response of BSA:AuNCs in 15% SEBS composite. Data generated without the use of sandwich pressure cell setup.**
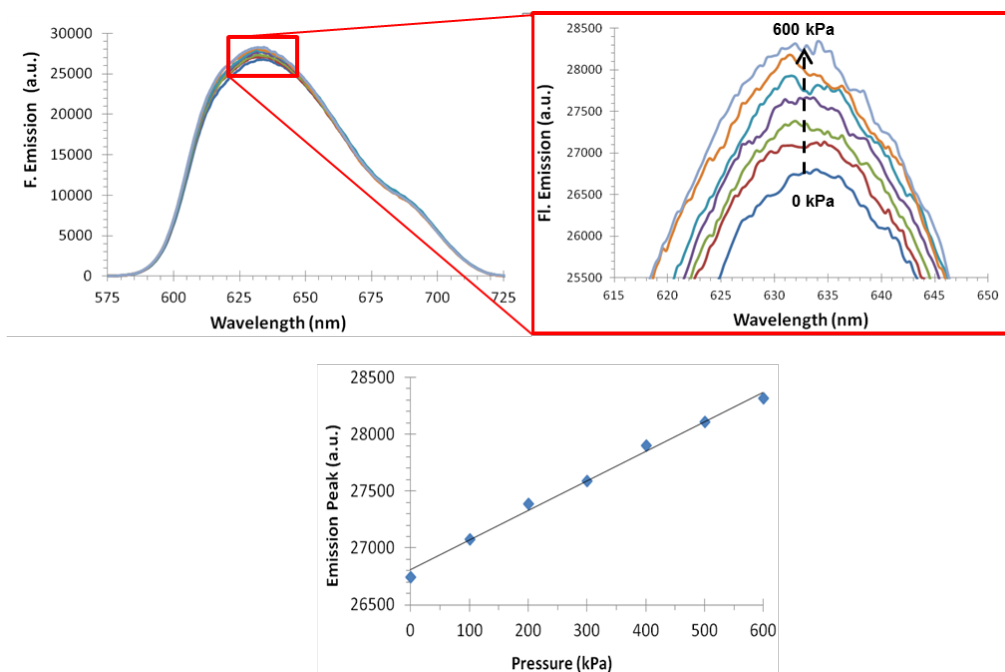
We hypothesize that the results observed above 2 MPa can either be attributed to a leakage of the 15% SEBS sandwich material past the mandrel and out of the cell, thus resulting in a loss of pressure or a quenching interaction between the mineral oil in the SEBS gel and the BSA:AuNCs. Mineral oil based fluorescence quenching is further supported when the 5% SEBS data for BSA:AuNCs is analyzed (Fig. 2c). In this data set there is no initial positive response, only a consistent linear decrease upon pressure addition. The 5% SEBS is much softer in modulus so that the 15% SEBS sample and has 10% higher mineral oil content. Thus, it is much easier for the mineral oil in the 5% gel to contact and quench the BSA:AuNC fluorescence. Future work will focus on quantifying their pressure response in stiffer gels to determine if there is indeed a relationship between pressure response and mineral oil content. Furthermore, we are also redesigning the pressure cell to ensure that no loss of the sandwich material occurs upon pressurization. We intend to overcome this challenge by using a much stiffer sandwich gel (i.e., 30% SEBS as opposed to the 15% SEBS that was used in this study).

QDs suspended in SEBS were used as a control to make sure these trends were not due to the experimental setup. These changes seen in QDs further support the theory that quenching is taking place in the BSA:AuNC-SEBS composites and is due to a unique interaction between the mineral oil in the composite and the BSA:AuNCs, as there is no significant change in fluorescence with the QD control.

4.   Summary and Conclusions

We have studied the effects of pressure on fluorescence every 50 lb_f or 0.78 MPa up to 400 lb_f or 6.24 MPa. Through the use of a sandwich structure and an acrylic load cell we were able see that several variables need to be addressed before any tissue injury conclusions can be determined from this type of study. More optimization is needed to fully understand the effect of pressure on the P:NC-SEBS composite systems. The data showed strong signs of quenching that appeared to be dependent upon the mineral oil content in the SEBS polymer composites. We predict that the higher mineral oil content paired with the softer modulus of the 5% SEBS gels leads to more direct interaction between the mineral oil and the BSA:AuNCs. This effect is not observed until above 2 MPa in the 15% SEBS composite, as higher pressures are needed in the stiffer gel to force interaction between the mineral oil and protein stabilized nanocluster.

Future studies will focus on changing the SEBS polymer density and BSA:AuNC concentrations to determine the effect of mineral oil content on the system. In addition, improvements should be made to the setup including the addition of a pressure sensor onto the BSA layer to directly measure pressure. Due to the pressure being measured at the top of the mandrel it is hard to quantify the loading on the BSA itself, except to say that it is under the maximum load experienced by the mandrel. A more robust load cell and using the outlined optimized testing procedure would be of great use since it would enable researchers to go above 8 MPa and explore the effect of much larger pressures on the system and compare it to previously published data. Different optics would allow testing to be done on pure BSA as well, thus leading to better controls. An enclosed, dark box setup would give much more control over many of the variables that can cause experimental error in the system and would give much more reliable results.

In conclusion, we have successfully synthesized ARL-developed brain mimicking polymer/BSA:AuNC composites and studied their pressure response in the low MPa regime. The composites exhibited modulus-based pressure response, wherein the stiffer composite showed a positive pressure response up to 2 MPa, and whereas the more pliable composite did not show a pressure response.

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List of Symbols, Abbreviations, and Acronyms

ARL	US Army Research Laboratory
AuNC	gold nanocluster
BSA	bovine serum albumin
BSA:AuNC	bovine serum albumin:gold nanocluster
IR	infrared
NSRDEC	US Army Natick Soldier RD&E Center
PNC	polymer gold nanocluster
QD	quantum dot
SEBS	styrene-ethylene/butylene-styrene
UV	ultraviolet

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