



ARL-TR-7572 • JAN 2016



Stabilizing Protein Effects on the Pressure Sensitivity of Fluorescent Gold Nanoclusters

by Abby L West, Mark H Griep, Christopher Knoblauch, and Shashi P Karna

Approved for public release; distribution is unlimited.

NOTICES

Disclaimers

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Citation of manufacturer's or trade names does not constitute an official endorsement or approval of the use thereof.

Destroy this report when it is no longer needed. Do not return it to the originator.



Stabilizing Protein Effects on the Pressure Sensitivity of Fluorescent Gold Nanoclusters

by Abby L West, Mark H Griep, and Shashi P Karna
Weapons and Materials Research Directorate, ARL

Christopher Knoblauch
Oak Ridge Institute for Science and Education, Oak Ridge, TN

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) January 2016		2. REPORT TYPE Final		3. DATES COVERED (From - To) May 2014–September 2015	
4. TITLE AND SUBTITLE Stabilizing Protein Effects on the Pressure Sensitivity of Fluorescent Gold Nanoclusters				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Abby L West, Mark H Griep, Christopher Knoblauch, and Shashi P Karna				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) US Army Research Laboratory ATTN: RDRL-WMM-A Aberdeen Proving Ground, MD 21005-5059				8. PERFORMING ORGANIZATION REPORT NUMBER ARL-TR-7572	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT This report describes the effect of scaffold protein secondary structure on the pressure response of protein-stabilized gold nanoclusters (P:NCs). These studies were conducted using an ethanol-driven pressure cell incorporated into a spectrofluorometer to measure the pressure sensitivity of P-NCs in real time. Results demonstrate that the pressure response of P:NCs is indeed dependent on the secondary structure of the protein. Proteins with high beta sheet content such as pepsin and DNase do not show a pressure response in the range studied (0–400 MPa), whereas fully alpha helical proteins like bovine serum albumin show linear pressure response over the full range. Interestingly, proteins low in secondary structure and high in random coil (i.e., lysozyme, 60% coil) show linear pressure response at pressures ranging up to 200 MPa but no response at higher pressures.					
15. SUBJECT TERMS nanotechnology, protein-stabilized nanoclusters, pressure response, pressure cell, hydrostatic, fluorescence					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 20	19a. NAME OF RESPONSIBLE PERSON Abby West
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (Include area code) 410-306-4952

Contents

List of Figures	iv
List of Tables	iv
Acknowledgments	v
1. Introduction and Background	1
2. Synthesis of Materials	2
2.1 Synthesis of BSA-Stabilized Gold Nanoclusters	2
2.2 Synthesis of Lysozyme-Stabilized Gold Nanoclusters	3
2.3 Synthesis of Pepsin-Stabilized Gold Nanoclusters	3
2.4 Synthesis of DNase-Stabilized Gold Nanoclusters	3
2.5 Ethanol-Driven Hydrostatic Pressure Cell	3
3. Results and Discussion	4
4. Summary and Conclusions	7
5. References	9
List of Symbols, Abbreviations, and Acronyms	11
Distribution List	12

List of Figures

Fig. 1	Crystal structures of proteins used in this study	2
Fig. 2	A) Fluorescence emission spectra of P:NCs upon excitation at 488 nm and B) P:NCs under brightfield illumination (top) and UV illumination (bottom). (left to right) BSA, lysozyme, DNase 1, and pepsin AuNCs.	4
Fig. 3	Pressure response of P:NCs tested: A) fully alpha helical BSA:AuNCs, B) random-coil-dominated lysozyme:AuNCs, C) beta-sheet-dominated, pepsin:AuNCs, and D) beta-sheet-dominated DNase:AuNCs.....	5

List of Tables

Table 1	Secondary structure content of the 4 proteins studied	2
Table 2	Pressure response for the various P:AuNCs tested in this study	6

Acknowledgments

This research was supported in part by an appointment to the Research Participation Program at the US Army Research Laboratory (ARL) administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and ARL. We would also like thank Alexis Fakner and Travis TumLin for their assistance and thoughtful consideration.

INTENTIONALLY LEFT BLANK.

1. Introduction and Background

Protein-stabilized nanoclusters (P:NCs) were first synthesized in 2009 using gold (Au) ions and bovine serum albumin (BSA) as both the stabilizing and reducing agent.¹ Since then P:NCs have been synthesized using several different proteins with varied structure and function.² These proteins range from the fully alpha helical serum protein BSA^{3–7} to the enzymes such as lysozyme^{8,9} and DNase 1,¹⁰ which are composed mostly of random loops and coils and high beta sheet content, respectively. In recent years P:NCs have been shown to have an increased level of tailorability through the use of different metals such as silver, copper, or platinum and by controlling the cluster size through reaction conditions.^{4,11–14}

An interesting attribute of P:NCs is that NC synthesis does not affect protein function, but the fluorescence of the P:NCs is largely affected by the environment of the stabilizing protein, allowing these hybrid systems to act as sensors in many applications.^{2,9,14–19} This has led to the creation of a multitude of P:NC-based sensors that use the chemical interactions of the stabilizing protein and its environment to detect the presence and concentration of a wide variety of substances including heavy metals and biological molecules.¹⁹ However, although a plethora of applications have been created to use the functional behavior of the stabilizing proteins of P:NCs to monitor chemical properties of the environment, there has been very limited research into using P:NCs to monitor mechanical/structural effects such as pressure response.

In 2013, Zhang et al.²⁰ conducted the first study on the response of P:NCs to mechanical forces by studying the pressure response of BSA: Au nanoclusters (AuNCs) inside a diamond anvil cell. BSA: AuNCs exhibited a linear increase in fluorescence intensity proportional to the pressure increase in the gigapascal (GPa) regime. More recently, our group conducted a study of BSA: AuNCs using a fluorimeter-based ethanol-driven pressure cell to study lower pressures from 0 to 400 MPa.²¹ However, these results are limited to the behavior of BSA: AuNCs and do not address if this is a widespread phenomenon among P:NCs or if the observed pressure response is limited to BSA-stabilized clusters. BSA is a unique protein; it fully comprises alpha helices, or springs. Thus, the observed pressure response is not surprising, as the protein is highly compressible.

In this investigation the effect of secondary structure composition on pressure response of red-emitting P: AuNCs was studied using 4 proteins of varied secondary structure elements (see Table 1 and Fig. 1). Results demonstrate that the pressure response of P:NCs is indeed dependent on the secondary structure of the protein. Proteins with high beta sheet content such as pepsin and DNase do not show a

pressure response in the range studied (0–400 MPa), whereas fully alpha helical proteins like BSA show linear pressure response over the full range. Interestingly, proteins low in secondary structure and high in random coil (i.e., lysozyme, 60% coil) show linear pressure response at pressures ranging up to 200 MPa but no response at higher pressures.

Table 1 Secondary structure content of the 4 proteins studied

Protein	α -helix (%)	B-sheet (%)	Turn (%)	Extended chain (%)
BSA	67	0	10	23
Lysozyme	30	13	27	30
DNase	40	34	12	12
Pepsin	3.4	63.1	7.3	26.2

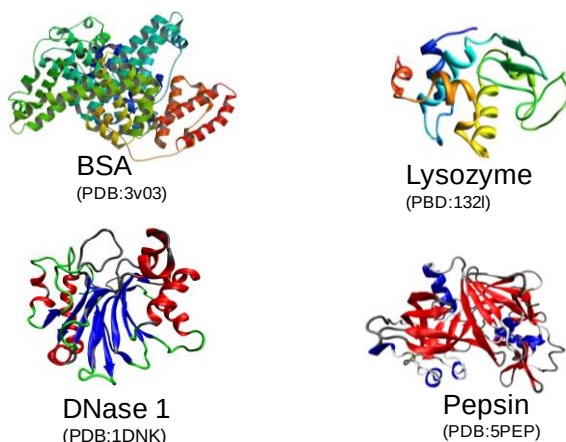


Fig. 1 Crystal structures of proteins used in this study

2. Synthesis of Materials

2.1 Synthesis of BSA-Stabilized Gold Nanoclusters

A solution of BSA:AuNCs was synthesized using published methods.¹ Aqueous chloroauric acid (HAuCl₄) solution (5 mL, 10 mM) was added to BSA solution (5 mL, 50 mg/mL) and stirred vigorously for 2 min. Sodium hydroxide (NaOH) solution (0.5 mL, 1 M) was subsequently added to bring the pH of the solution to 12. The solution was then incubated at 37 °C overnight while stirring until the solution turned a dark brown color. The BSA:AuNCs solution was then spin-purified using a 5-kDa MWCO spin filter several times to remove excess Au salt. The purified sample was lyophilized and resuspended at a concentration of 10 mg/mL in ultrapure water.

2.2 Synthesis of Lysozyme-Stabilized Gold Nanoclusters

A solution of lysozyme:AuNCs was synthesized using published methods.⁹ Aqueous HAuCl₄ solution (5 mL, 5 mM) and lysozyme (5 mL, 10 mg/mL) were added to 5 mL of ultrapure water and stirred vigorously for 5 min. NaOH solution (0.5 mL, 1 M) was subsequently added to bring the pH to 12 and, the solution was incubated at 37 °C overnight while stirring. The solution was then spin-purified using a 5-kDa MWCO spin filter several times to remove excess Au salt. The purified sample was lyophilized and resuspended at a concentration of 10 mg/mL in ultrapure water.

2.3 Synthesis of Pepsin-Stabilized Gold Nanoclusters

A solution of pepsin:AuNCs was synthesized using published methods.²² Aqueous HAuCl₄ solution (5 mL, 5 mM) was added to pepsin solution (5 mL, 40 mg/mL) while stirring vigorously. NaOH solution (1.5 mL, 1 M) was subsequently added to bring the pH to 12, and the solution was incubated at 37 °C while stirring for 4 h. The solution was then spin-purified using a 5-kDa MWCO spin filter several times to remove excess Au salt. The purified sample was lyophilized and resuspended at a concentration of 10 mg/mL in ultrapure water.

2.4 Synthesis of DNase-Stabilized Gold Nanoclusters

A solution of DNase:AuNCs was synthesized using published methods.¹⁰ Aqueous HAuCl₄ solution (5 mL, 5 mM) was added to DNase 1 solution (5 mL, 20 mg/mL) while stirring vigorously. NaOH solution (0.5 mL, 1 M) was subsequently added to bring the pH to 12, and the solution was incubated at 37 °C while stirring for 12 h. The solution was then spin-purified using a 5 kDa MWCO spin filter several times to remove excess Au salt. The purified sample was lyophilized and resuspended at a concentration of 10 mg/mL in ultrapure water.

2.5 Ethanol-Driven Hydrostatic Pressure Cell

See *Use of an Ethanol-Driven Pressure Cell to Measure Hydrostatic Pressure Response of Protein-Stabilized Gold Nanoclusters*²¹ for complete instructions for the operation of the ethanol-driven hydrostatic pressure cell. The P:NC solutions were each resuspended in ultrapure water at a concentration of 10 mg/mL for hydrostatic pressure response analysis.

3. Results and Discussion

P:NCs with bright red fluorescence were successfully synthesized using BSA, lysozyme, DNase 1, and pepsin (see Figs. 2A and 2B). The measured emission spectra show peaks ranging from 630 to 650 nm for all P:NCs. These spectra are consistent with the fluorescence spectra of 25-atom P:NCs found in literature.^{1,9,10,17,21}

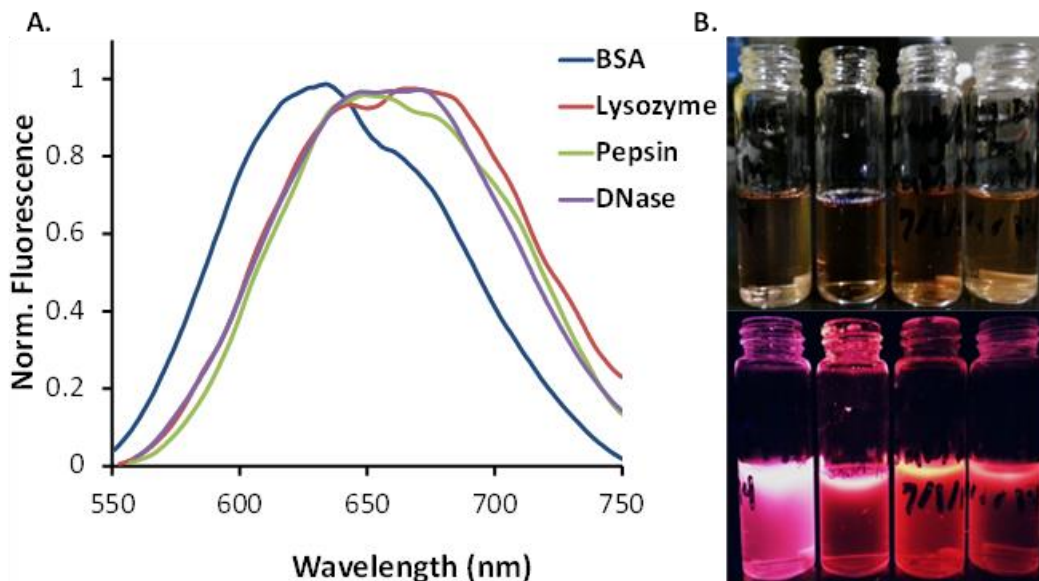


Fig. 2 A) Fluorescence emission spectra of P:NCs upon excitation at 488 nm and B) P:NCs under brightfield illumination (top) and UV illumination (bottom); (left to right) BSA, lysozyme, DNase 1, and pepsin AuNCs

The pressure dependence of BSA:, lysozyme:, DNase 1:, and pepsin:AuNC fluorescence was characterized using an ethanol-driven pressure cell from 0 to 400 MPa. The effect of secondary structure on pressure response was then characterized for the 4 different P:NCs. These results are summarized in Fig. 3.

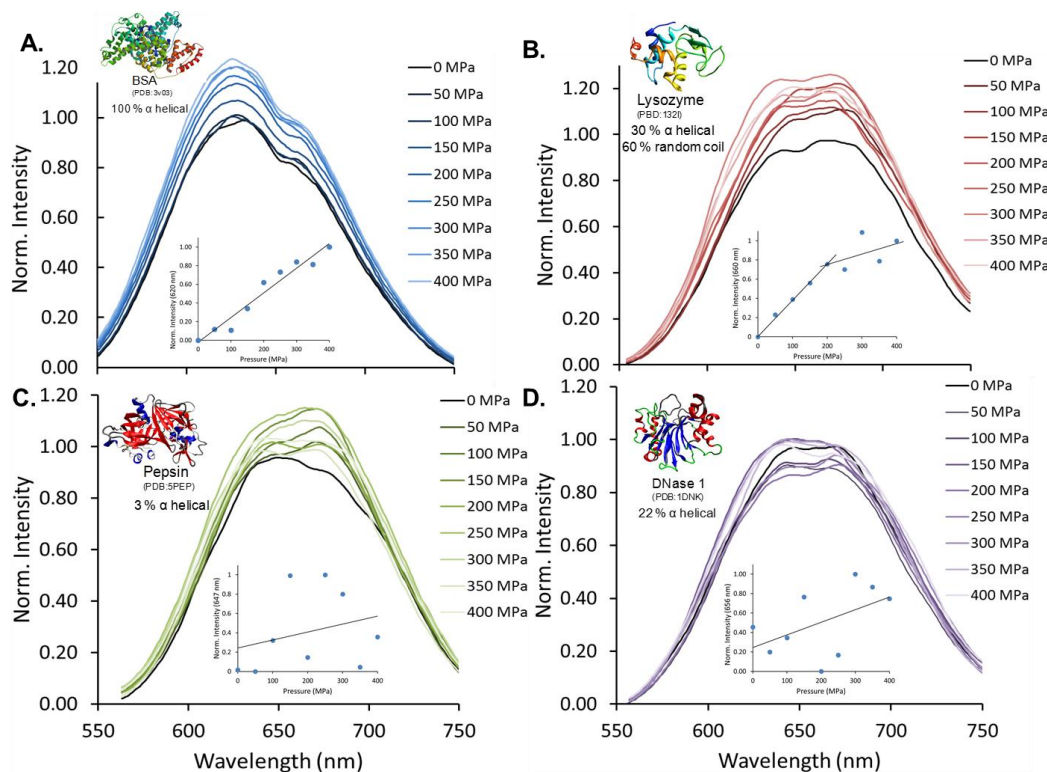


Fig. 3 Pressure response of P:NCs tested: A) fully alpha helical BSA:AuNCs, B) random-coil-dominated lysozyme:AuNCs, C) beta-sheet-dominated pepsin:AuNCs, and D) beta-sheet-dominated DNase:AuNCs

BSA- and lysozyme-stabilized AuNCs exhibit linear pressure-induced fluorescence enhancement, whereas pepsin- and DNase-stabilized AuNCs do not exhibit any pressure response in the investigated pressure range. Interestingly, while BSA:AuNCs maintain a linear pressure response over the full range studied (0–400 MPa), lysozyme:AuNCs exhibit a dramatic decrease in pressure response at pressures higher than 150 MPa.

Although the exact mechanisms of this change in pressure response are currently unknown; the flexible element (alpha helix, extended coil and turn) content of the stabilizing proteins could play a major role. Table 1 highlights the much higher flexible element content of BSA and lysozyme—100% and 87%, respectively—than DNase (64%) and pepsin (37%), which may explain why the latter 2 P:NCs show a negligible pressure response.

Understanding the role secondary structure plays in pressure response may be simplified if the secondary structure elements are thought of as common objects. Alpha helices can be thought of as highly compressible springs, extended chains and turns are like floppy strings, and beta sheets are like inflexible boards. The pressure response observed for BSA:AuNCs was hypothesized to be due to the

compression of protein ligands around the AuNC, thus leading to enhanced electronic density and higher fluorescence. In our study, the intensity of the fluorescence peak of the BSA:AuNCs exhibits a significant linear relationship with applied pressure. This results in an increase in fluorescence intensity of approximately 23% at 400 MPa. BSA is composed almost entirely of alpha helices. It is logical that the protein would show a linear response over a wide pressure range (0–2 GPa, combining our study and the study conducted by Zhang et al.²⁰), as the protein's spring-like structure would be able to compress quickly and continuously over a wide range of pressure.

Interestingly, the percentage of turn and extended chain structural components for lysozyme comprises more than half (57%) of the secondary structure elements of the protein. The higher extended chain content found in lysozyme may be attributed to the steep pressure dependence observed from 0 to 150 MPa, as, stated *vide supra*, the extended coil and turn elements are similar to strings in that they are very quickly and easily compressed to their noncompressible/final state. The fact that lysozyme has nearly twice the random coil content as BSA correlates with the pressure response of the lysozyme:AuNCs under 150 MPa, which is approximately twice as high as that of the BSA:AuNCs, at 0.1%/MPa versus 0.06%/MPa, respectively, as shown in Table 2. As the random coil structure is more mechanically compliant than the α -helical and β -sheet structures, one might hypothesize that they would dominate the low-pressure response of the P:NCs.

Table 2 Pressure response for the various P:AuNCs tested in this study

Protein	Pressure response (%/MPa)	R ²
BSA	0.06	0.94
Lysozyme <150 MPa	0.10	0.94
Lysozyme >150 MPa	0.04	0.36
Pepsin	0.02	0.28
DNase	0.01	0.082

Note: R² = coefficient of determination.

Looking at pressures above 150 MPa, it can be seen that a dramatic decrease occurs in the pressure response of the lysozyme:AuNCs. In this high-pressure region, the BSA:AuNCs maintain a linear pressure response of 0.06%/MPa while the lysozyme:AuNCs exhibit a significant drop in pressure response to 0.04%/MPa. Again, the exact mechanisms of this behavior are unknown and will require further study. However, it can be seen in Table 1 that this behavior seems to correlate with the percentage of α -helical structure in the protein. BSA, which has a very high percentage of α -helical structure, exhibits a much higher pressure response in this region than the lysozyme:AuNCs. The pepsin:AuNCs, which have virtually no α -helical structure, exhibit a negligible pressure response in this region. The fact

that the mixed α - β DNase:AuNCs do not exhibit pressure response in this region despite having a significant percentage of α -helical structure suggests that the pressure response in this pressure region is a function of the ratio of α -helix and β -sheet structure present in the stabilizing protein.

These data support the hypothesis that the pressure-induced fluorescence enhancement experienced by P:NCs is influenced by the mechanical structure of the stabilizing protein. The high random coil content of lysozyme gives it a much more compliant structure than BSA. This would explain the very high response of lysozyme:AuNCs compared with the BSA:AuNCs. The drop in pressure response of the lysozyme:AuNCs at pressures above 150 MPa can likely be attributed to the complete collapse of the lysozyme structure at very high pressures, as opposed to the stiffer BSA structure, which can maintain its linear pressure response at these higher pressures.

4. Summary and Conclusions

In this study, the influence of the secondary structure of protein-stabilized nanoclusters, or P:NCs, on the pressure-induced fluorescence enhancement was investigated. Red-emitting AuNCs composed of 25 atoms were synthesized using a variety of stabilizing proteins with various secondary structural properties and subjected to increasing hydrostatic pressures in the range of 0–400 MPa using an ethanol-driven pressure cell. The influence of the pressure on the fluorescence emission peak was measured using a spectrofluorometer at 50-MPa intervals.

It was found that BSA- and lysozyme-stabilized AuNCs exhibit pressure-induced fluorescence enhancement in the range of 0–400 MPa while DNase- and pepsin-stabilized AuNCs exhibit a negligible pressure response. It was also found that the lysozyme:AuNCs showed a significant difference in pressure response when comparing pressures lower and higher than 150 MPa.

These data demonstrated that the secondary structure of the stabilizing protein has a major effect on the pressure-induced fluorescence enhancement of the P:NCs. The results indicate that at pressures lower than 150 MPa the random coil component of the protein structure dominates the pressure response, while at pressures higher than 150 MPa the pressure response is dominated by a function of the α -helix and β -sheet structure, with a higher-percentage α -helical structure yielding a higher-pressure response.

The results found in this study have helped illuminate the effect of protein structure on the pressure response of P:NCs. However, further testing will need to be completed to get a full understanding of the effect of protein structure on pressure-induced fluorescence enhancement. This testing will include examining the fluorescence response of a wider variety of protein structures as well as examining the changes to the protein structure directly as a response to pressure using Fourier transform infrared approaches used in previous work.²⁰

Once the effects of protein structure on the pressure response of P:NCs is understood, investigations into the effect of various metals and nanocluster sizes can be initiated. This information will allow for a high degree of control over the pressure response of synthesized P:NCs, resulting in a suite of potential pressure monitoring devices for various pressure applications.

Future work will also include an investigation into the effect of embedding the pressure-sensitive P:NCs into a clear polymer matrix. Although it has been determined that P:NCs retain their fluorescent properties when embedded in a polymer matrix (data not shown), the pressure response of these embedded P:NCs has yet to be investigated.

The results of this preliminary investigation are very promising and show that not only do P:NCs exhibit a linear pressure-induced fluorescence enhancement, but that this pressure response can be tuned by varying the secondary structure of the stabilizing protein.

5. References

1. Xie J, Zheng Y, Ying JY. Protein-directed synthesis of highly fluorescent gold nanoclusters. *J Am Chem Soc.* 2009;131:888–889.
2. Chevrier DM, Chatt A, Zhang P. Properties and applications of protein-stabilized fluorescent gold nanoclusters: short review. *J Nanophotonics.* 2012;6:1–16.
3. Aswathy B, Sony G. Cu²⁺ modulated BSA-Au nanoclusters: a versatile fluorescence turn-on sensor for dopamine. *Microchem J.* 2014;116:151–156.
4. Guo CL, Irudayaraj J. Fluorescent Ag clusters via a protein-directed approach as a Hg(II) ion sensor. *Analytical Chemistry.* 2011;83:2883–2889.
5. Hemmateenejad B, Shakerizadeh-shirazi F, Samari F. BSA-modified gold nanoclusters for sensing of folic acid. *Sensor Actuat B-Chem.* 2014;199:42–46.
6. Hu D, Sheng Z, Gong P, Zhang P, Cai L. Highly selective fluorescent sensors for Hg(2+) based on bovine serum albumin-capped gold nanoclusters. *Analyst.* 2010;135:1411–1416.
7. Hu L, Han S, Parveen S, Yuan Y, Zhang L, Xu G. Highly sensitive fluorescent detection of trypsin based on BSA-stabilized gold nanoclusters. *Biosens Bioelectron.* 2012;32:297–299.
8. Joseph D, Geckeler KE. Synthesis of highly fluorescent gold nanoclusters using egg white proteins. *Colloids Surf B Biointerfaces.* 2014;115:46–50.
9. Wei H, Wang ZD, Yang LM, Tian SL, Hou CJ, Lu Y. Lysozyme-stabilized gold fluorescent cluster: synthesis and application as Hg²⁺ sensor. *Analyst.* 2010;135:1406–1410.
10. West AL, Griep MH, Cole DP, Karna SP. DNase 1 retains endodeoxyribonuclease activity following gold nanocluster synthesis. *Anal Chem.* 2014;86:7377–7382.
11. Chen D, Zhao C, Ye J, Li Q, Liu X, Su M, Jiang H, Amatore C, Selke M, Wang X. In situ biosynthesis of fluorescent platinum nanoclusters: toward self-bioimaging-guided cancer theranostics. *ACS Appl Mater Interfaces.* 2015;7:18163–18169.

12. Gao SP, Chen DH, Li QW, Ye J, Jiang H, Amatore C, Wang XM. Near-infrared fluorescence imaging of cancer cells and tumors through specific biosynthesis of silver nanoclusters. *Sci Rep-UK*. 2014;4:4384–4389.
13. Mei-Na Su JY, Qi-Wei Li, Shui-Hong Li, Wei Ge, Xiao Chen, Hui Jiang, Xue-Mei Wang. Novel in situ biosynthesized fluorescent zinc nanoclusters for specific cellular bio-imaging. *Chinese Chemical Letters*. 2015;26(11):1400–1402.
14. Qu XL, Yichen Li, Lei Wang, Y, Liang J, Liang J. Fluorescent gold nanoclusters: synthesis and recent biological application. *J Nanomaterials*. 2015;6:1–23.
15. Liu CL, Wu HT, Hsiao YH, Lai CW, Shih CW, Peng YK, Tang KC, Chang HW, Chien YC, Hsiao JK, Cheng JT, Chou PT. Insulin-directed synthesis of fluorescent gold nanoclusters: preservation of insulin bioactivity and versatility in cell imaging. *Angew Chem Int Edit*. 2011;50:7056–7060.
16. Shang L, Dong SJ, Nienhaus GU. Ultra-small fluorescent metal nanoclusters: synthesis and biological applications. *Nano Today*. 2011;6:401–418.
17. Wen F, Dong YH, Feng L, Wang S, Zhang SC, Zhang XR. Horseradish peroxidase functionalized fluorescent gold nanoclusters for hydrogen peroxide sensing. *Analytical Chemistry*. 2011;83:1193–1196.
18. Xie J, Zheng Y, Ying JY. Highly selective and ultrasensitive detection of Hg(2+) based on fluorescence quenching of Au nanoclusters by Hg(2+)-Au(+) interactions. *Chem Commun (Camb)*. 2010;46:961–963.
19. Zhang LB, Wang EK. Metal nanoclusters: new fluorescent probes for sensors and bioimaging. *Nano Today*. 2014;9:132–157.
20. Zhang M, Dang YQ, Liu TY, Li HW, Wu YQ, Li Q, Wang K, Zou B. Pressure-induced fluorescence enhancement of the BSA-protected gold nanoclusters and the corresponding conformational changes of protein. *J Phys Chem C*. 2013;117:639–647.
21. West AL, Griep MH, Knoblauch C, Karna, SP. Use of an ethanol-driven pressure cell to measure hydrostatic pressure response of protein-stabilized old nanoclusters. Aberdeen Proving Ground (MD): Army Research Laboratory (US); 2015; in press.
22. Kawasaki H, Hamaguchi K, Osaka I, Arakawa R. pH-dependent synthesis of pepsin-mediated gold nanoclusters with blue green and red fluorescent emission. *Adv Funct Mater*. 2011;21:3508–3515.

List of Symbols, Abbreviations, and Acronyms

Au	gold
AuNC	BSA: Au nanocluster
BSA	bovine serum albumin
GPa	gigapascal
HAuCl ₄	chloroauric acid
NaOH	sodium hydroxide
P:NC	protein-stabilized nanocluster

1 DEFENSE TECHNICAL
(PDF) INFORMATION CTR
DTIC OCA

2 DIRECTOR
(PDF) US ARMY RESEARCH LAB
RDRL CIO LL
IMAL HRA MAIL & RECORDS
MGMT

1 GOVT PRINTG OFC
(PDF) A MALHOTRA

3 DIR USARL
(PDF) RDRL WM
S KARNA
RDRL WMM A
M GRIEP
A WEST