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Water-soluble luminescent nanoparticles have been fabricated with suitable surface chemistry to attach biomolecules, engineered to attach via spontaneous self-assembly. The resulting bio-conjugates have been characterized and the effects of pH and ionic strength on stability examined. Additionally, methods to improve bacterial expression and purification of the fusion proteins have been developed. QDs coated with protein G, which binds antibody, have been utilized in developing sensitive immunoassays. Finally, viral capsids, which are made of repeating subunits, are being utilized as signal amplifiers, enhancing the sensitivity of optical sensors with the goal of detecting single molecular events

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FINAL REPORT

GRANT #: N00014-01-WX-21008

PRINCIPAL INVESTIGATOR: George P. Anderson Ph.D.

INSTITUTION: Naval Research Laboratory

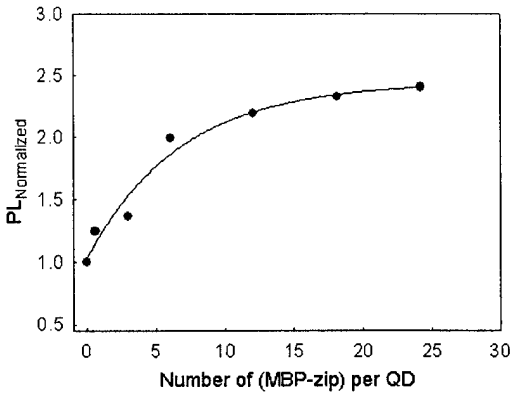
GRANT TITLE: Luminescent Nanoparticles for High Sensitivity Biosensing

AWARD PERIOD: 1 JUNE 1999 - 31 DECEMBER 2000

OBJECTIVE: Combine the newly available luminescent nanoparticles with custom designed recombinant fusion proteins to provide a means to achieve ultra-sensitive biodetection for hazardous agents or pollutants. An additional effort will examine the use of viral capsid as material for nanotechnology.

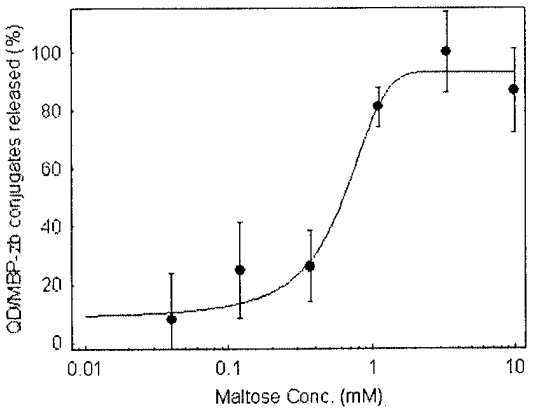
APPROACH: Water-soluble luminescent nanoparticles will be fabricated having suitable surface chemistry to attach biomolecules, engineered to attach via spontaneous self-assembly. The resulting bio-conjugates will be characterized and the effects of pH and ionic strength on stability examined. Additionally, methods to improve bacterial expression and purification of the fusion proteins will be developed. QDs coated with protein G will be utilized in developing sensitive immunoassays. Finally, viral capsids, which are made of repeating subunits, will be utilized as signal amplifiers, enhancing the sensitivity of optical sensors and detection of single molecular events

ACCOMPLISHMENTS: We have prepared water-soluble luminescent nanoparticles, which maintain their properties for many months. We have also successfully cloned and isolated two different fusion proteins capable of self-assembling on the surface of QDs. These proteins, maltose-binding protein (MBP) and Protein G, both fused to a basic leucine zipper, were found to enhance the quantum yield of the water soluble quantum dots, and retain their luminescence for several months. The enhancement in luminescence upon binding of the protein was found to be dose dependent.



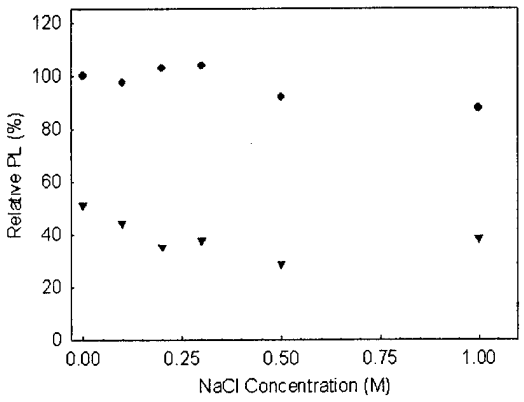
1. PL enhancement occurring upon bioconjugate formation at increasing MBP-zb:QD ratios (data normalized vs. unconjugated QDs).

The resulting saturation curve agreed well with the theoretical packing estimate of approximately 19 molecules for each dot. In addition the QD/protein assembly was found to be stable under physiological conditions, while the enhancement in luminescence increased with pH. Experiments have been conducted that show the proteins retain their native activity when attached to the surface of the quantum dots. Using the QD/MBP-zb bound to amylose beads we have been able to analyze samples for maltose at sensitivities less than 100 nM.



2. Release of QD/MBP-zb bioconjugates from amylose beads as a function of concentration of added maltose.

Most importantly these complexes were found to be quite stable after formation. Even salt concentration as high as one molar failed to disrupt the interaction. The stability of the interaction between the QDs and recombinant fusion protein has been one of the keys to preparing highly active materials. Another feature that proved critical has been the use of the QD/MBP-zb conjugates in conjunction with QDs coated with Protein G- zb. By preparing mixed surfaces, we were able to use the function of the MBP to bind the QDs to the amylose resin, while any excess antibody not bound by the Protein G- zb is simply washed away. These dots are now being shown to be capable of binding antibody and assays have confirmed that those antibodies can be used in the performance of immunoassays



3. Effect of increasing ionic strength on the photoluminescence of CdSe-ZnS QD/MBP-zb conjugates (■) and unconjugated QDs (●).

The studies involving viral capsid have utilized self-assembly of Cow Pea Mosaic Virus (CPMV) to form complex patterns. CPMV was isolated from cowpea plants in yields of 1-2 grams per kg of leaves. 2μL droplets of diluted CPMV solutions were placed on freshly cleaved mica and acid-treated mica. The self-assembled patterns of virus particles

formed during the drying on surfaces were examined using an Olympus optical microscope and a Nanoscope III atomic force microscope (AFM).

At a low concentration (0.0015 mg/ml), individual CPMV particles on freshly cleaved mica can be seen in Figure. 4b. It is known that the average virus diameter is 30 nm in diameter. The measured height of virus particles was found to be 27.0 ± 0.4 nm which is consistent with the size of the virus particle. This suggests that the virus particles are not compressed by the tip force employed for scanning or distorted during the drying process.

At a higher concentration of 0.15 mg/ml CPMV, virus particles self-assemble into macroscopic structures with distinct morphologies during the drying of concentrated CPMV droplets on surfaces. An AFM image of droplet, which was dried on freshly cleaved mica, shows that the virus particles organize into parallel and orthogonal lines, forming finger-like patterns. The high-resolution AFM images show that these parallel fingers have almost same height of 250 nm, which is close to 9 virus layers. The average width of the fingers is about 600 ± 40 nm. The typical distance between the parallel fingers is about 7 μm. The self-assembly of virus particles during the drying can be mediated by surface properties. Freshly cleaved mica was treated with 0.5 M HCl for 2 hrs followed by a 30 min wash with ultra pure water. It has been demonstrated that OH groups can be created at the acid treated mica surface. Unlike the finger-like structures seen on freshly cleaved mica, the virus particles self-assemble into cross-like structures. Simple hydrodynamic effects can explain these variations in the morphology of the structures, depending on the surface conditions, with the contact line pinning as the critical boundary condition.

CONCLUSIONS: We extensively tested our initial recombinant fusion protein, the MBP-bz. It was shown to easily and stably coat DHLA-coated QDs. These dots were found to have their luminescence increased significantly. This initial fusion protein continues to be an intergral part of our research efforts, since it allows a facial method of separating protein bound to the dot surface from those free in solution. This is a critical and often difficult step in the preparation of coated nanospheres. This method proved to be sufficiently novel and deemed important enough to seek patent protection. Since these initial beginnings we have also prepared a Protein G-zb. Protein G binds antibodies and can be used to create a generic luminescent particle for use in immunoassays. The Protein G-zb has been shown to also bind to QDs, increase the QD luminescence, and be functional for use in immunoassays. The results of these experiments are reported elsewhere, see PUBLICATIONS.

The studies involving viral capsid have utilized self-assembly of Cow Pea Mosaic Virus (CPMV) to form complex patterns. This material may find applications in formation of nano to micro scale patterning.

SIGNIFICNACE: We are developing new types of nanosensors by combining the unique properties of semiconductor nanocrystals with biologically derived (or bio-inspired) molecular recognition elements. Our work is of broad interest to interdisciplinary materials nanoscience, to biological and chemical molecular sensing, and to the physics of nanostructures. Successful implementation of our goals will be important to both the Navy and the Nation in developing a new generation of low cost/low maintenance sensors and maintaining our stature as leaders in the developing nanotechnology arena.

PATENT INFORMATION: "Inorganic Particle Conjugates," United States Patent Application, G. P. Anderson, H. Mattoussi, J. M. Mauro, M. G. Bawendi, and V. C. Sundar, MIT Case No. 8733, Navy Case. No. 82,333, U.S. Patent Application Serial No. 60/190,766, filed March 20, 2001

AWARD INFORMATION: None

PUBLICATIONS

1. Mattoussi, H, Mauro, JM, Goldman ER, Green T, Anderson GP, Sundar VC, Bawendi MG, (2000) "Bioconjugation of higly luminescent colloidal CdSe-ZnS quantum dots with an engineered two-domain recombinant protein" phys. stat. sol. (b) 224: 277-283.

2. Mattoussi H, Mauro JM, Goldman ER, Anderson GP, Sundar VC, Milulec FV, Bawendi MG (2000) "Self-assembly of CdSe-ZnS quantum dot bioconjugates using an engineered recombinant protein" J. Am. Chem. Soc. 122: 12142-12150.

3. Tran, P. T., Goldman, E. R., Mattoussi, H., Anderson, G. P., and Mauro, J. M. (2001) "Bioconjugates of luminescent CdSe-ZnS quantum dots with an engineered two-domain protein G for use in fluoroimmunoassays," in Nanoparticles and Nanostructured Surfaces: Novel Reporters with Biological Applications, Catherine J. Murphy, Ed., Proceedings of SPIE vol. 4258, pp. 1-7 (2001).

4. Goldman, E. R., Mattoussi, H., Tran, P. T., Anderson, G. P., and Mauro, J. M. (2001) "Bioconjugates of luminescent CdSe-ZnS quantum dots with engineered recombinant proteins: Novel self-assembled tools for biosensing," in Semiconductor Quantum Dots, S. Fafard, D. Huffaker, R. Leon, and R. Noetzel, Editors, Mater. Res. Soc. Proc., Pittsburgh, 2001, 642, J2.8.1-J2.8.6.