Hamilton Undergraduate Scholarship Programme 2010

PROJECT NO. 2

Supervisor: Dr. Enrico Marsili
Co-supervisors: N/A

Project Title: Microbial biosynthesis of gold nanoparticles (AuNPs)

Duration of Project **From:** 7 June 2010 **To:** 13 August 2010

Project Description:

This project aims to synthesize AuNPs from the bioreduction of Au^{3+} in purified cell-free extract of the fungus *Rhizopus oryzae.* AuNPs are relevant to design of biosensors for chemical and bacterial contaminants.

1.Background & preliminary work - Metal nanoparticles has been studied for their applications in bioimaging and biosensors.[1] Nanoparticles size and shape strongly influence their optical and electronic characteristics.[2] Shape control of gold nanostructures enable fine tuning of their optical properties in various detection strategies.[2,3] Gold nanoparticles (AuNPs) are usually synthesized by reducing a gold salt with sodium citrate or sodium borohydride, followed by surface modification of the produced particles with capping ligands and organic solvents that often raises environmental questions.[2,4]

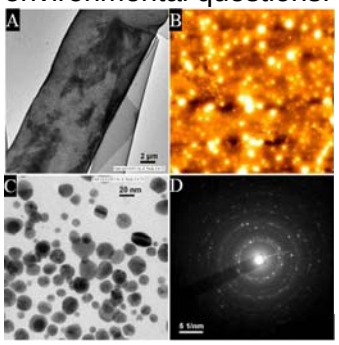

Figure 1: Transmission Electron Microscopy (TEM) (A) and Atomic Force Microscopy (AFM) (B) show the formation of gold nanoparticles on the surface of the fungal mycelia. High Resolution TEM (HRTEM) (C) shows nanoparticles without noticeable aggregation. The Selected Area Electron Diffraction (SAED) pattern (D) indicates that the synthesized gold nanoparticles are single crystals.

Microorganisms are capable of synthesizing inorganic nanomaterials.[5] Understanding the biochemical processes involved in such synthesis is appealing as an environmentally friendly alternatives to chemical methods for nanoparticle synthesis.[6] Currently, there is little understanding of the conditions which control nanoparticles size and shape. Capping with natural amino acids has been recently used to synthesize nanoparticles of given structures.[7] However, the complexity of the biological system renders the analysis and identification of active species in the nucleation and growth of metal nanoparticles a daunting task. Preliminary experiments (Fig. 1) shows the synthesis of single crystalline AuNPs using both fungal mycelia of *Rhizopus oryzae* and its cell-free extract.[8]

2. Experimental work - *R. oryzae* will be grown to stationary phase at 30 ºC. Supernatants will be collected by centrifugation and the bioreduction of gold ions (Au^{+3}) to AuNPs (Au^{0}) will be monitored through UV-vis spectrophotometer by appearance of absorbance band at 540 nm. The role of cell viability in AuNPs synthesis will be assessed by using heat inactivated or microorganisms as control

experiment. The enzymatic reduction of the gold ion to metallic gold will be carried out by cell-free extract of the organism. Following growth, cells will be harvested from the culture medium, washed and resuspended in buffer. Cells will be lysed through French Press[2]. The lysate will be centrifuged to remove debris and remove membrane-associated material, thereby obtaining generating the soluble extract. As it has been postulated that intracellular enzymes are mainly responsible for synthesis of AuNPs,[5] the soluble extract will be purified through ammonium sulphate fractionation, anion-exchange chromatography, chromatofocusing, and gel filtration. Purified enzymes will be used for synthesis of AuNPs as previously described.[8]

3. References

1. Han, M.; Gao, X.; Su, J. Z.; Nie, S. *Nature Biotechnol*. **2001,** *19***,** 631.
2. Nikoobakht, B.; El-Sayed, M. A. *J. Phys. Chem. A* **2003**, *107*, 3372.
3. Jin, R. C.; Cao, Y. W.; Mirkin, C. A.; Kelly, K. L.; Schatz, G. C.; Zheng, J. G. *Science* **2001**, *294*, 1901.
4. Niemeyer, C. M.; Burger, W.; Peplies, I. *Angew. Chem.*, *Int. Ed.* **1998**, *37*, 2265.
5. Kröger, N.; Deutzmann, R.; Sumper, M. *Science* **1999**, *286*, 1129.
6. Bazylinski, D. A.; Frankel, R. B. *Nature Rev. Microbiol.* **2004**, *2*, 217.
7. Shankar, S. S.; Rai, A.; Ankamwar, B.; Singh, A.; Ahmad, A.; Sastry, M. *Nature Mater.* **2004**, *3*, 482.
8. Das SK, Marsili E. A green chemical approach for the synthesis of gold nanoparticles: characterization and mechanistic aspect. *Rev. Environ. Sci. BioTech*. DOI 10.1007/s11157-010-9188-5.

4. Role and responsibilities of the exchange student -The undergraduate student will be supervised on a day-to-day basis by Dr. Sujoy K. Das, postdoctoral researcher in our group. In addition to the research duties described in section 2, the student will:

- Submit weekly report to the advisor, Dr. Enrico Marsili;
- Contribute to the submission of conference papers and journal articles.