



Interfacing Biology with Nanomaterials

Murali Sastry
Nanoscience Group
Materials Chemistry Division
National Chemical Laboratory, Pune - 411 008

E-mail : sastry@ems.ncl.res.in



Indo-US Meeting on Nanomaterials
11-13 August, 2004 (IISc Bangalore)



Making the connection between nanomaterials and biologicals

- Biological methods for the synthesis of inorganic nanoparticles
- Inorganic nanoparticles as scaffolds for immobilization of :
 - Amino acids (stabilization of nanoparticles; assembly of nanoparticles; amino acid-stabilized nanoparticles as templates for mineral growth)
 - Proteins/enzymes (reusable biocatalysts)
- 3) DNA/PNA (structural interconnects; nanoparticle assembly; application in biodiagnostics)
- Free-standing nanogold membranes

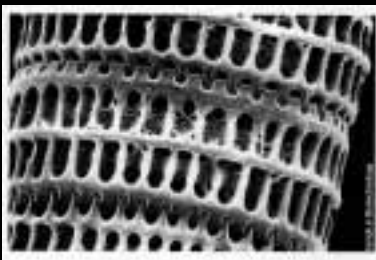


Biological nanosynthesis methods



Exquisite nanoscale/mesoscale structures in nature

Diatoms (marine organisms,
amorphous silica)



Magnetotactic bacteria
(magnetite nanoparticles)

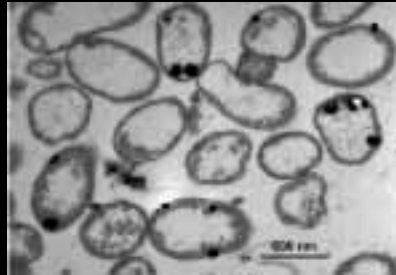


Biomimetic approach to advanced materials, particularly
nanomaterials.

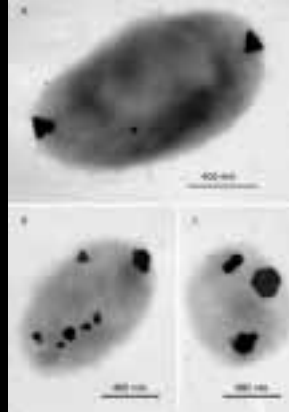




Biosynthesis of nano-silver using bacteria



Pseudomonas stutzeri extracted from silver mines



Klaus et al. PNAS 96 (1999) 13611.



If bacteria can synthesize metal nanoparticles, can plant organisms such as fungi do so ?

Can we do it extracellularly ?

What is the biochemical process leading to nanoparticle formation ?

Can we extend the methodology to other chemical compositions ? Sulphides ? Oxides ?

Is size control possible ? Better monodispersity ?

How about nanoparticle shape modulation ?

Can we use extracts of plants in nanobiosynthesis ?





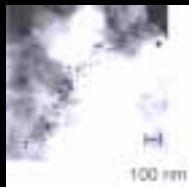
Intracellular nano-gold using *Verticillium* sp.



Verticillium biomass



Verticillium after reaction with AuCl_4^- ions



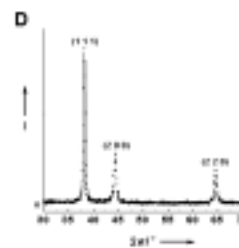
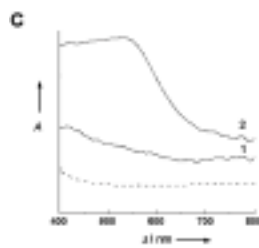
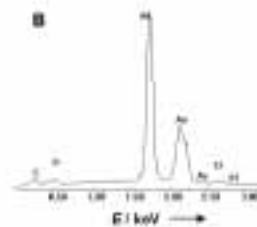
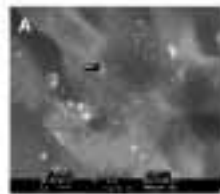
← TEM thin sections →



Angew.Chem.Int.Ed. 40 (2001) 2585 (nanogold)
Nano Lett. 1 (2001) 515 (nanosilver)



Intracellular nano-gold using *Verticillium* sp.

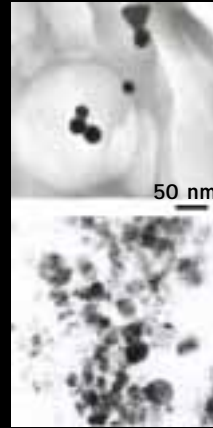
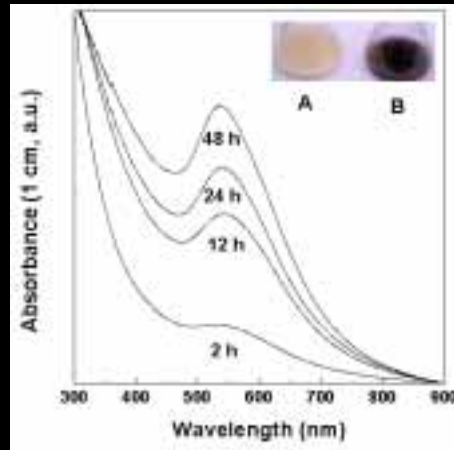


Angew.Chem.Int.Ed. 40 (2001) 2585 (nanogold)
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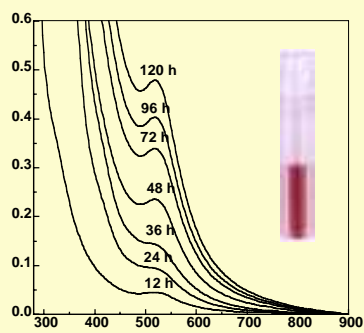
Extracellular nano-gold using *Fusarium oxysporum*



ChemBioChem 3 (2002) 461 (nanogold)
Colloids Surf.B. 28 (2003) 313 (nanosilver)



Extra-cellular biosynthesis of nano-gold using extremophilic actinomycetes (*Thermomonospora* sp.)



Size : 8 nm
Monodispersity :
better than 18 %

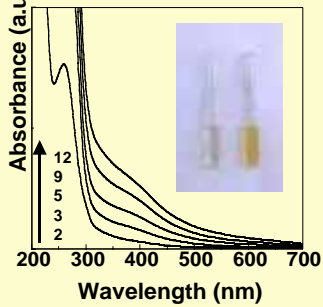


Langmuir 19 (2003) 3550.



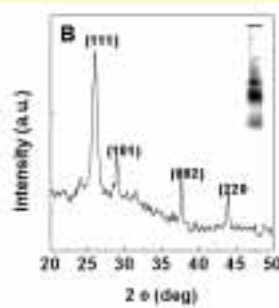


Biosynthesis of CdS Q-dots using fungi (extra-cellularly)

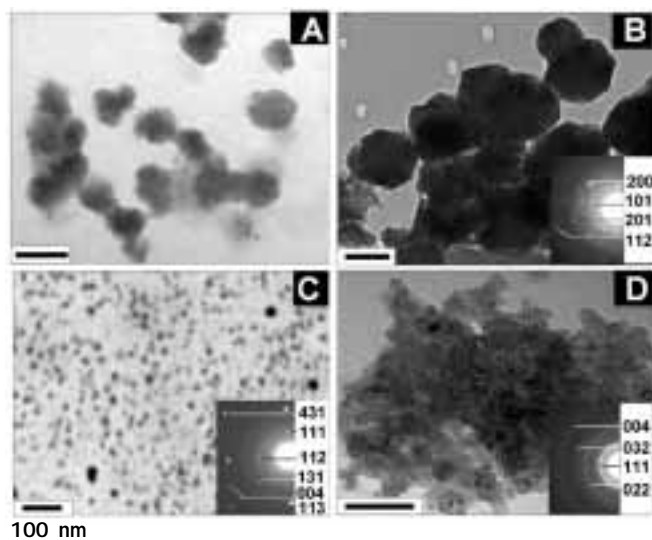


J. Am. Chem. Soc. **124** (2002) 12108.

Review : *Microbial nanoparticle production in Nanobiotechnology : Concepts, applications and perspectives*, Ed. by Mirkin & Niemeyer, Wiley-VCH, 2004, Ch. 9, 126.



Biosynthesis of silica and titania using a fungus.



Silica

Titania

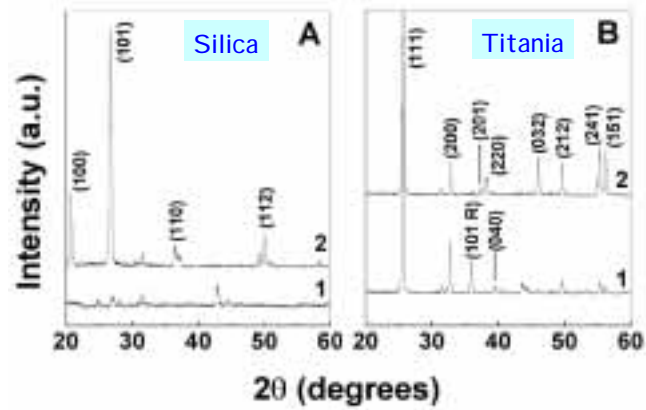
100 nm

Reaction of aqueous K_2TiF_6/K_2SiF_6 with the biomass

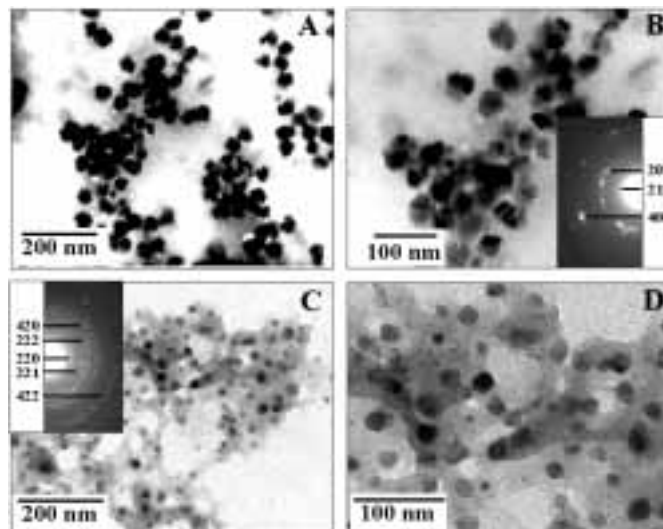




Biosynthesis of silica and titania using a fungus.

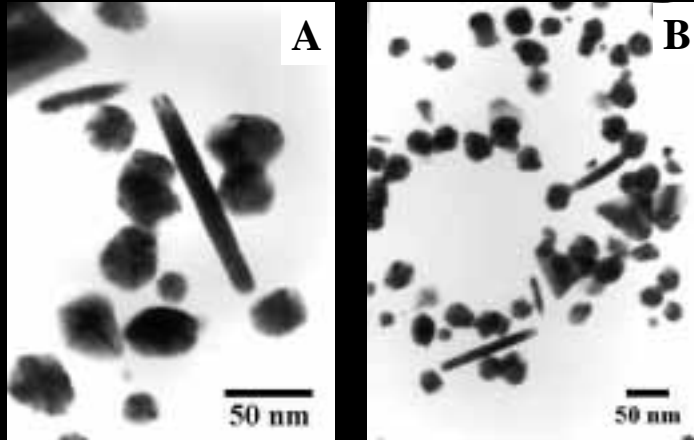


Biosynthesis of magnetite using a fungus.





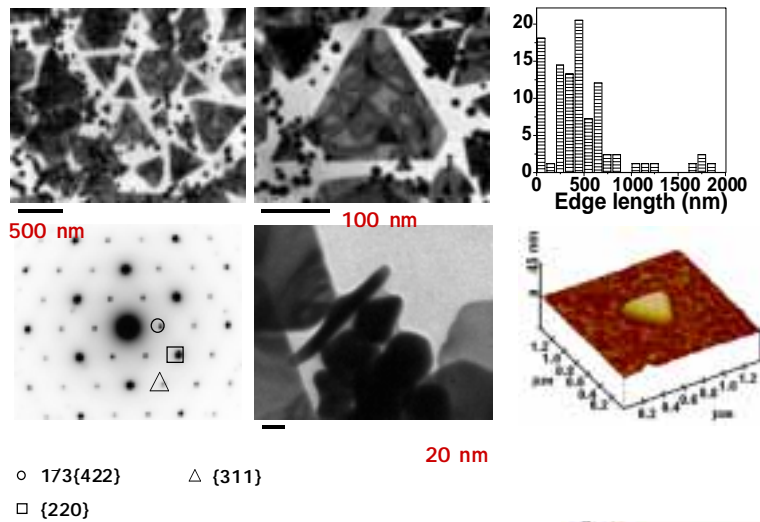
Gold nanoparticle synthesis using geranium leaves



J.Mater.Chem. 13 (2003) 1822.



Synthesis of triangular gold nanoparticles using plant extract

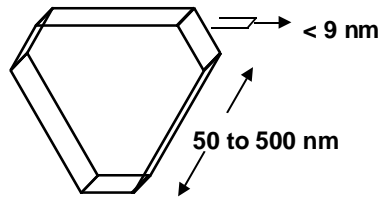
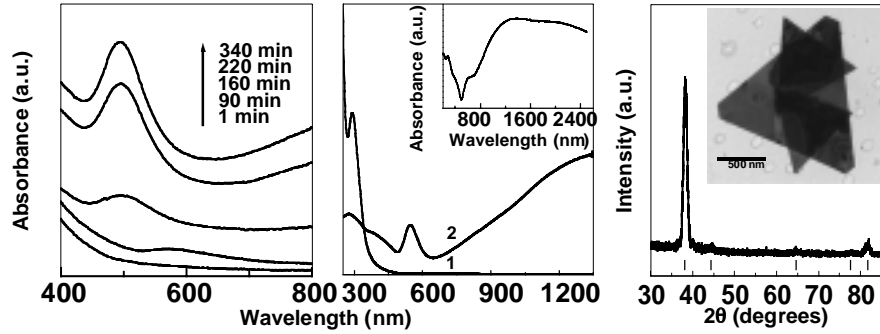


Nature Materials 3 (2004) 482-488.





Synthesis of triangular gold nanoparticles using plant extract



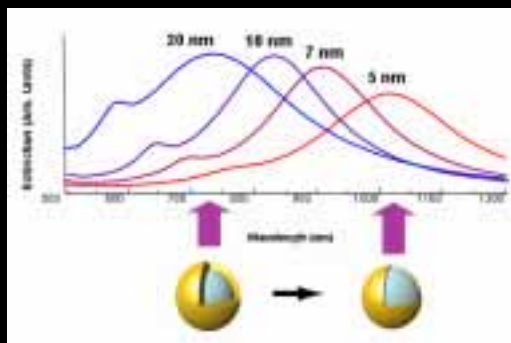
Nature Materials 3 (2004) 482-488

Potential applications :

- Hyperthermia of cancer cells
- Scanning probe microscopy tips
- Optical coatings (plasmonics ?)



Core-shell particles with NIR absorption : application in hyperthermia of cancer cells



Nanospectra Biosciences Inc.
Houston, Texas; www.nanospectra.com

Magnetic nanoparticles : Triton Biosystems

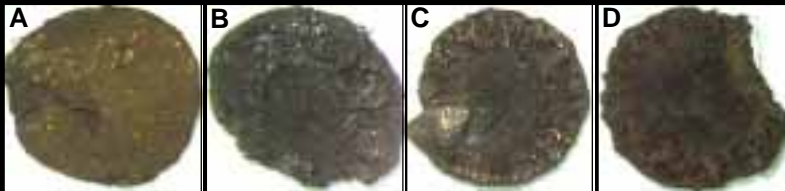




Free-standing nanogold membranes



CaCO₃ deposited on functionalized nanogold membranes

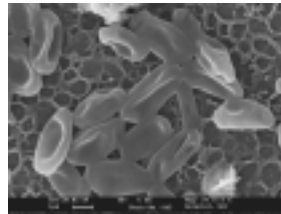
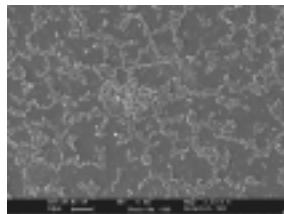


1 cm

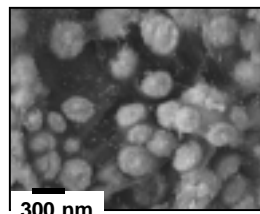
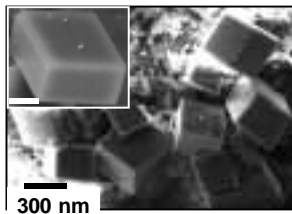


Free-standing nanogold membranes : applications

Cell immobilization (*Candida bombicola*) – organic transformations, bone implants



Biomaterial growth – bone implants



Chem.Mater. 16 (2004) 988 – CaCO₃ crystallization
Langmuir 20 (2004) 3717 – pepsin immobilization.





Isothermal Titration Calorimetry Studies on the Binding of Amino Acids to Gold Nanoparticles

J. Phy. Chem. B, 108 (2004) 11535-11540.



Binding of aspartic acid (acidic) and lysine (basic)
were studied with :

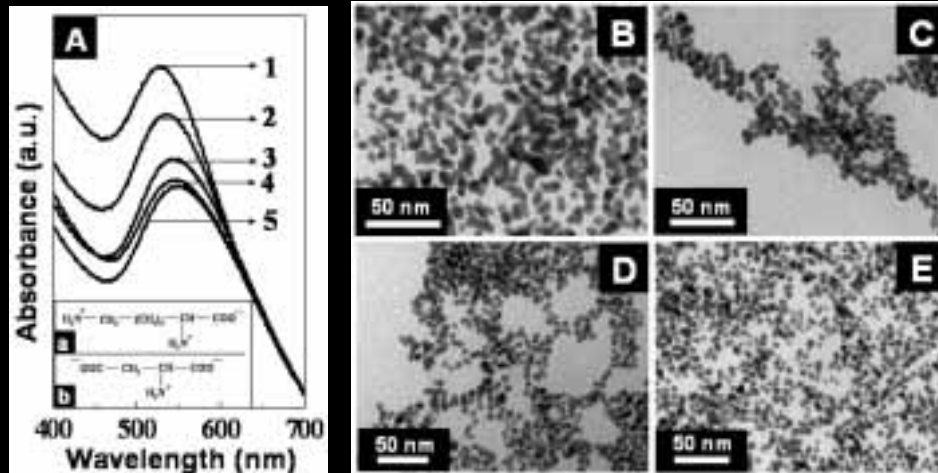
1. UV-Visible spectroscopy
2. Transmission electron microscopy
3. Isothermal titration calorimetry (ITC)
4. X-ray photoelectron spectroscopy
5. Gel electrophoresis

at different pH and at different concentrations of amino acid.





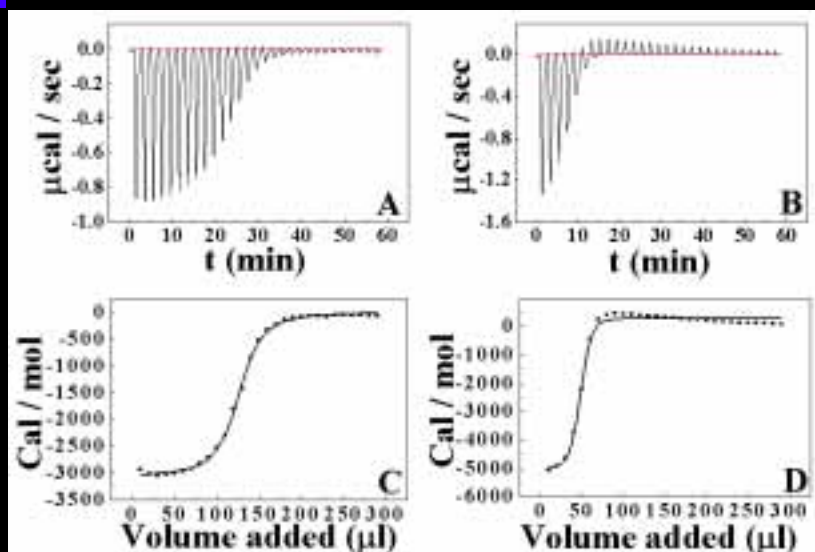
Optical properties and TEM analysis



- Optical properties Au -amino acid
- Au uncapped; C) Au-Aspartic acid
- D) Au-Lysine at pH 7; E) Au-Lysine at pH 11



Thermograms of Au-aspartic acid at different amino acid concentrations

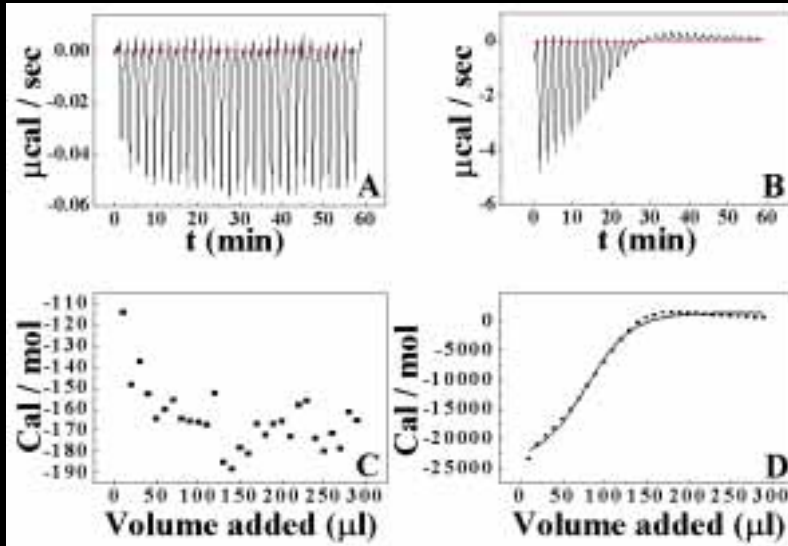


- A: Au-Aspartic acid (10^{-3} M) at pH 7
- B: Au-Aspartic acid (2×10^{-3} M) at pH 7





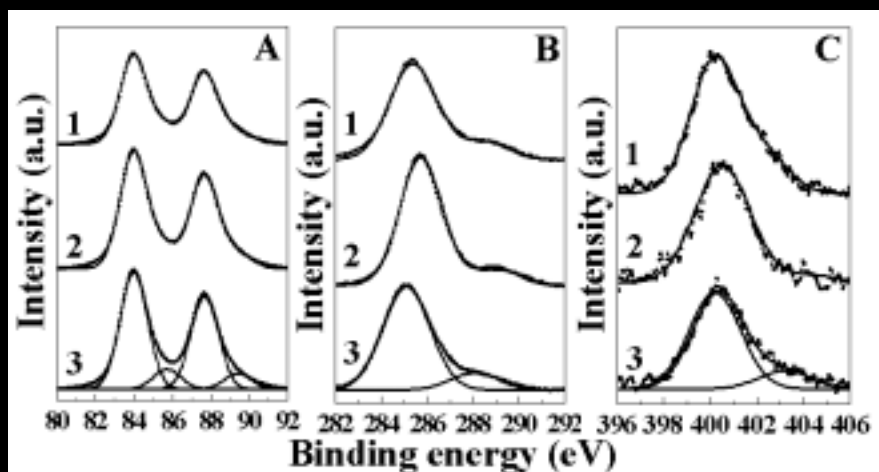
Thermograms of Au-lysine at different pH



A: Au-lysine (10^{-3} M) at pH 7
B: Au-lysine (10^{-3} M) at pH 11



XPS analysis of A : Au_{4f} B : C_{1s} C : N_{1s} core levels

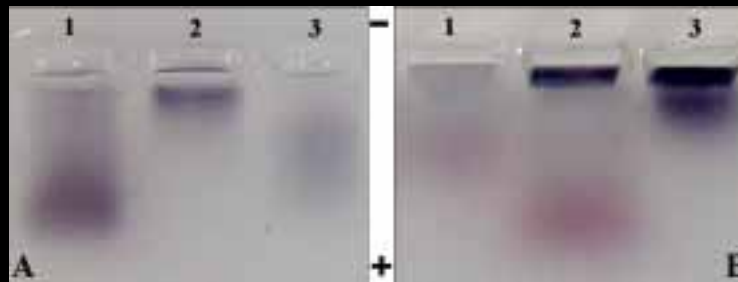


1) Au-lysine at pH 11; 2) Au-lysine at pH 7
3) Au-aspartic acid at pH 7





Electrophoretic mobility of Au-amino acids



- 1. Au-lysine at pH 11; 2. Au-lysine at pH 7; 3. Au uncapped
- 1. Partially capped Au-aspartic acid
2. Fully capped Au-aspartic acid; 3. Au uncapped

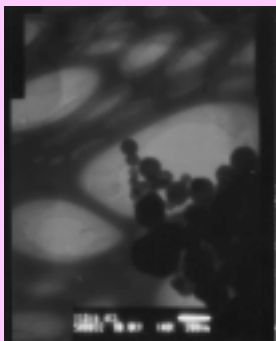


Gold nanoparticles for enzyme immobilization



Protein - nanoparticle bio-inorganic conjugate materials

Biocatalytic activity of pepsin-Au nanoparticle conjugates in water



TEM picture of pepsin-Au nanoparticle structures

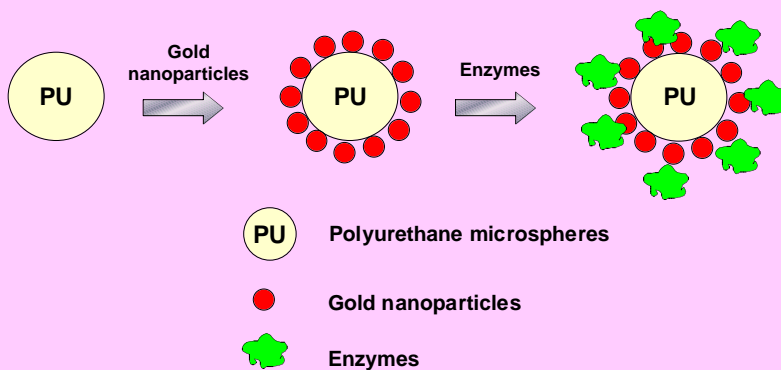
Time (in days)	Pepsin-Au conjugate Specific activity (%)	Pepsin specific activity (%)
0	100	100
1	72	43
2	25	18
3	10	3

Langmuir 17 (2001) 1674.



Polymer-Au nano bioconjugates

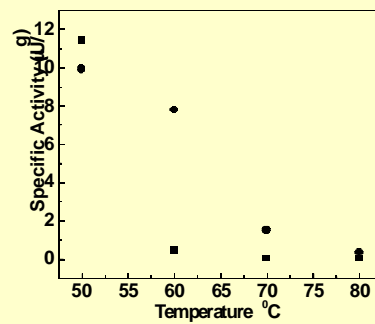
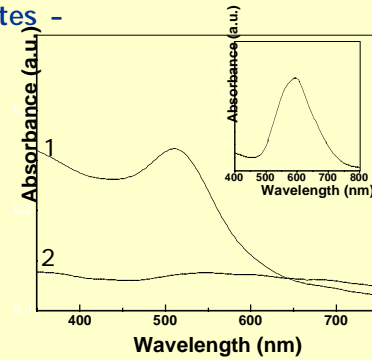
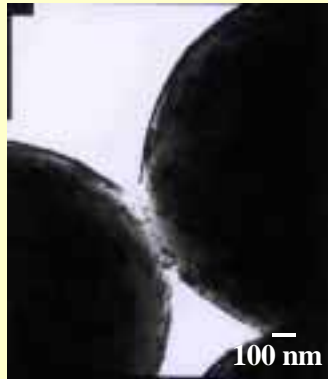
Scheme



Chem.Mater. 13 (2003) 15.



Polymer-Au nano bioconjugates -



Chem.Mater. 13 (2003) 15.



Table 1

Biocatalytic activities obtained from assaying the pepsin-gold nanoparticles-PU bioconjugate material for six sequential reuses.

System	Activity (IU/μg) [#]
Pepsin in solution	11.2
Pepsin-gold-PU spheres, run 1	13.2
Pepsin-gold-PU spheres, run 2	10.2
Pepsin-gold-PU spheres, run 3	8.0
Pepsin-gold-PU spheres, run 4	7.2
Pepsin-gold-PU spheres, run 5	4.3
Pepsin-gold-PU spheres, run 6	3.6

[#] One unit of protease activity is measured as a change in absorbance at 280 nm of 0.001 per minute at pH 2 and 37 °C measured as acid soluble products using casein as the substrate.

Chem.Mater. 13 (2003) 15.





Table 2

Biocatalytic activities obtained from assaying free pepsin in solution and the pepsin-nanogold-PU bioconjugate material pre-incubated at different pH for 1 h.

pH	Activity of free enzyme in solution (IU/ μ g) [#]	Activity of the enzyme in the bioconjugate material (IU/ μ g) [#]
2	11.2	13.2
5	10.7	8.5
8	0	5.2
10	0	0.3

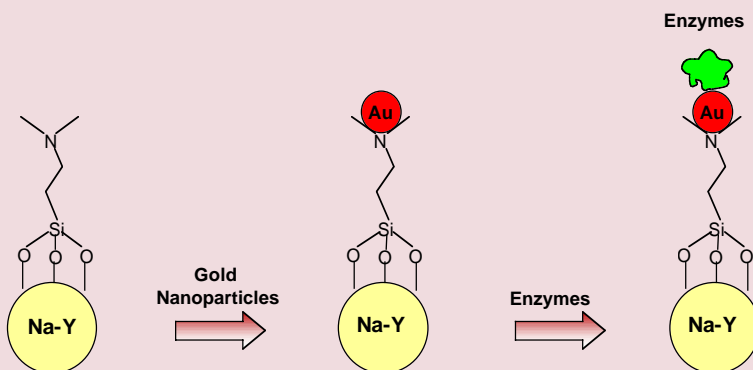
[#] One unit of protease activity is defined as a change in absorbance at 280 nm of 0.001 per minute at pH 2 and 37 °C measured as acid soluble products using casein as the substrate.

Chem.Mater. 13 (2003) 15.



Enzyme immobilization on amine-functionalized zeolites

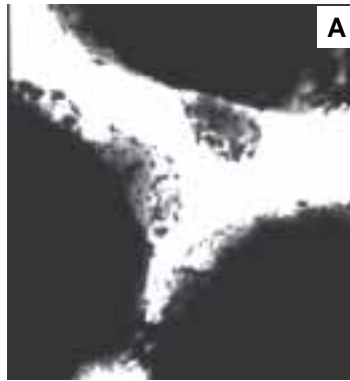
Scheme



Langmuir 19 (2003) 2858



TEM images of gold nanoparticles bound to amine functionalized Na-Y zeolites



100 nm

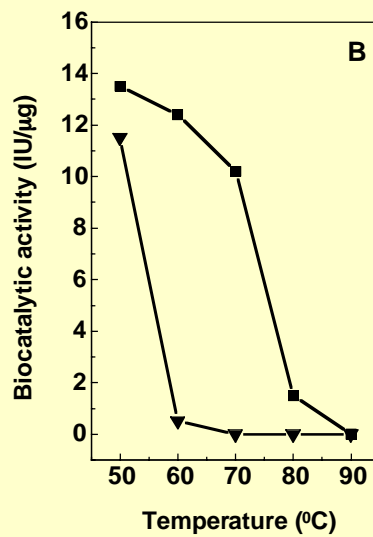
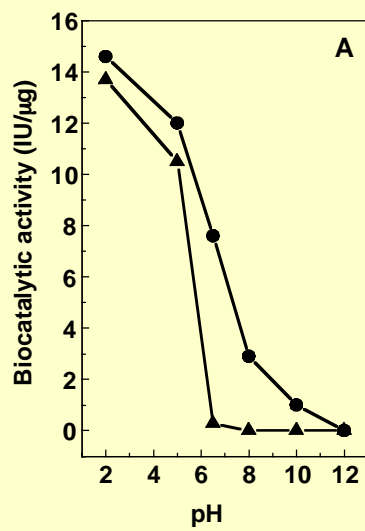


100 nm

Langmuir 19 (2003) 2858



Biocatalytic activity of F-prot-Au nano-zeolite



Langmuir 19 (2003) 2858





My group



Acknowledgment :

Dr. Rajiv Kumar Dr. Islam Khan Dr. A. Ahmad
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