Nanoparticle Synthesis and Assembly for Biological Diagnostics

Gil Lee

Schools of Chemical and Biological Engineering

Hao Shang and Wonsuk Chang

Templated Synthesis of Nanoparticles

Magnetic Nanoparticle Assembly

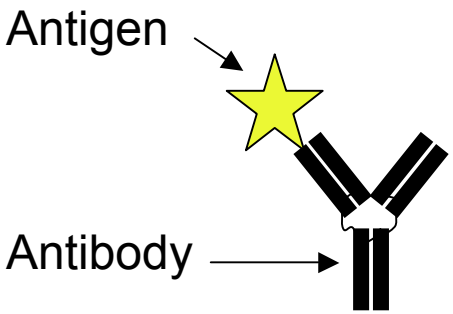
Support: Division of Infection Diseases, Eli Lilly Inc

NASA Institute for Nanoelectronics and Computing at Purdue

1.0 Biological Sensing: Immunoassays

Nanoparticles are currently used as *optical and chemical transducers* in many commercially available diagnostic kits.

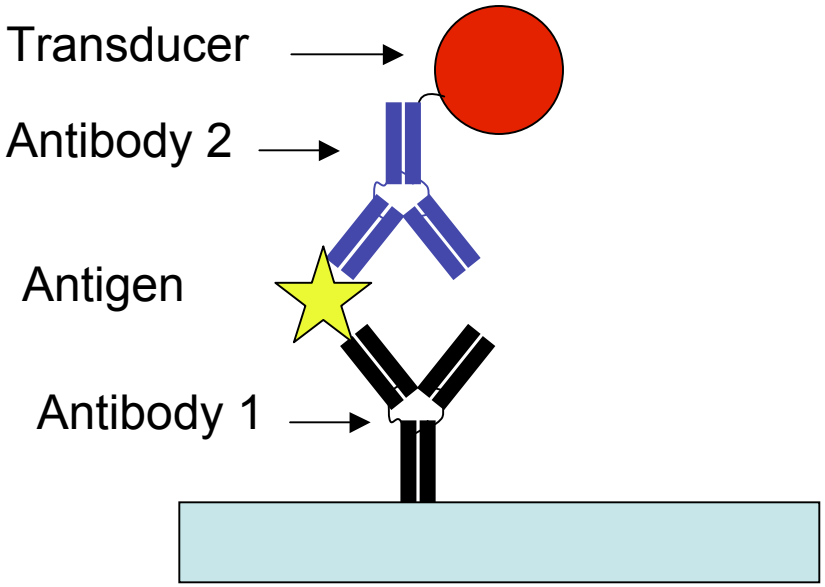
Antibodies are commonly used molecular receptors for these diagnostic assays:



$$Ab + Ag \underset{K_{off}}{\overset{K_{on}}{\rightleftharpoons}} Ab - Ag$$

$$Keq = \frac{K_{on}}{K_{off}} = \frac{[Ab - Ag]}{[Ab][Ag]} = \frac{10^{6} - 10^{8}}{10^{-4} - 1} = 10^{6} - 10^{9}$$

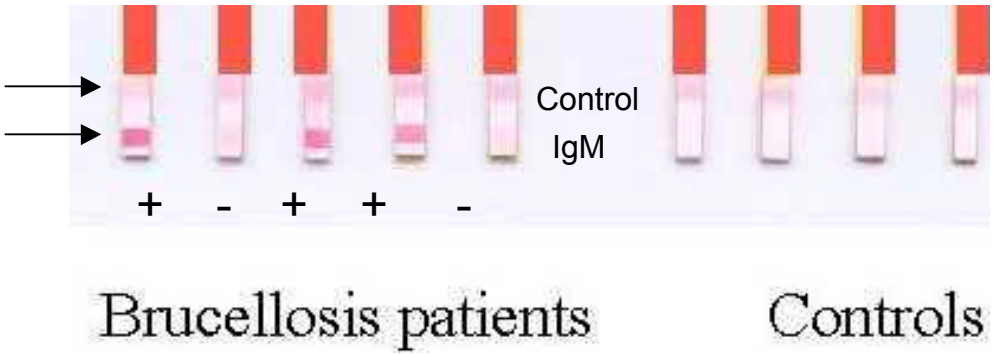
Solid Phase Sandwich Immunoassay:



Sensitivity is determined by both *Keq* and kinetics.

1.0 Biological Sensing: Immunogold Assays

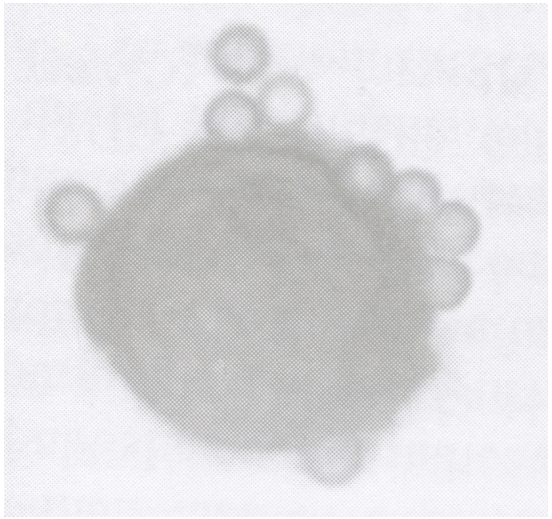
Dipstick assays use *antibody coated colloidal gold* to detect antigens



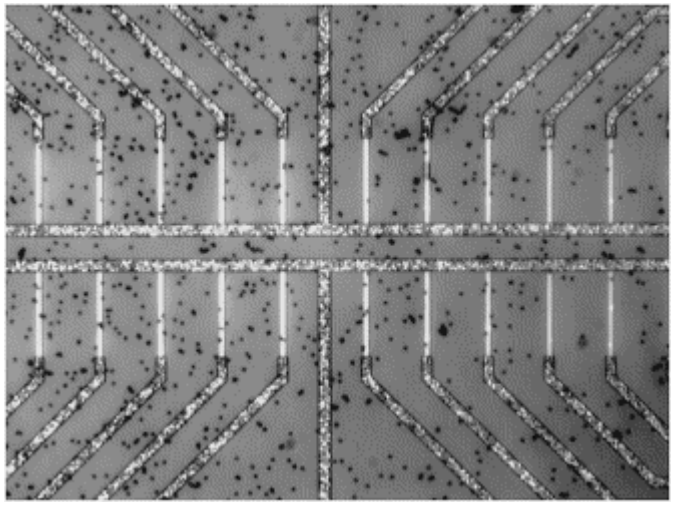
"The dipstick assay is aimed at the rapid detection of *Brucella*-specific IgM antibodies in human serum or whole blood samples from patients in the early stage of the disease. The dipstick assay is based on the binding of specific IgM antibodies to a lipolysaccharide fraction … The assay utilize stabilized non-enzymatic detection reagents that **can be stored without the need for refrigeration for at least two years without losing reactivity**. The result of the dipstick assay is **obtained after three hours and no special equipment is required to perform the assay**."

KIT - Royal Tropical Institute of the Netherlands. Assay format include Brucellosis, Leprosy, Leptospirosis, Leishmaniasis, and Typhoid Fever

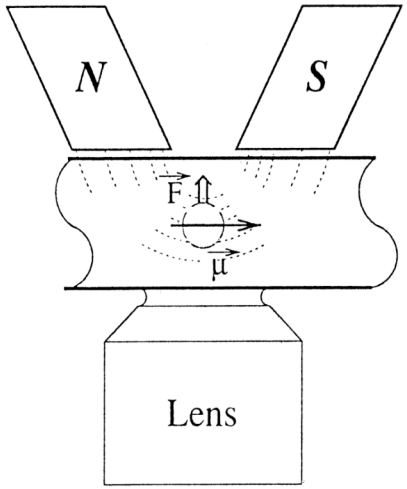
1.0 Biological Sensing: Magnetic Materials in Sensing



Magnetic Separation



Giant Magnetoresistance



Magnetic Tweezers

Prestvik WS etc. Scientific and Clinical Applications of Magnetic Carriers; Baselt DR. etc. Biosensors & Bioelectronics, 13, 371; Gosse and Croquette Biophysical Journal. 82, 3314

2.1 Physical Basis for Optical Properties of Nanoparticles

The optical properties of colloidal dispersions have been studied for several hundred years and have been described by rigorous theory:

Rayleigh Scattering

$$Q_{sca} = \frac{8x^4}{3}\left|\frac{m^2-1}{m^2+2}\right|^2 \propto \frac{1}{\lambda^4}$$

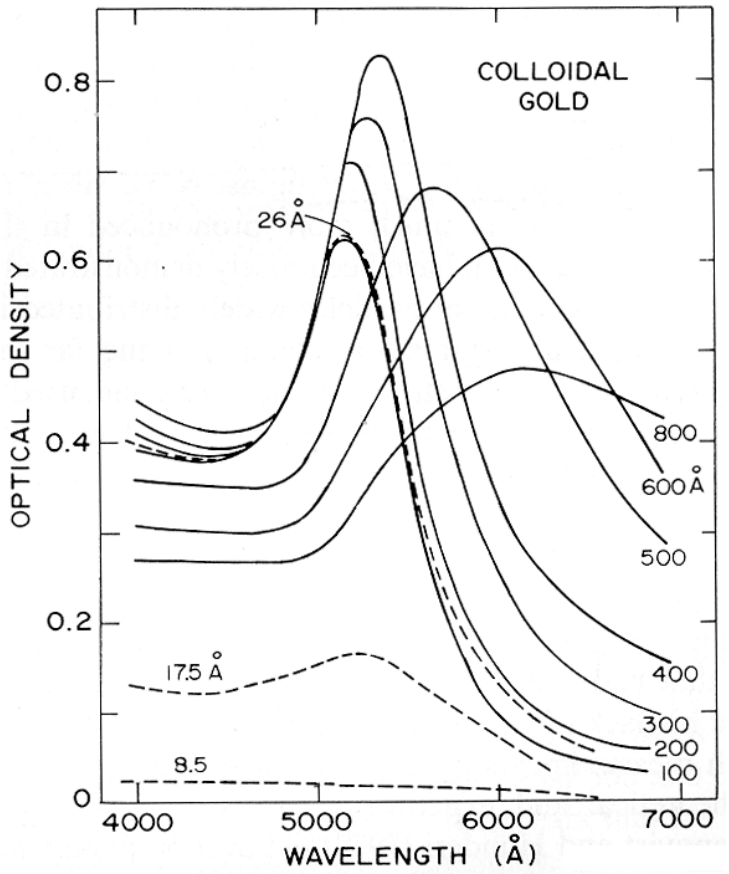
Adsorption

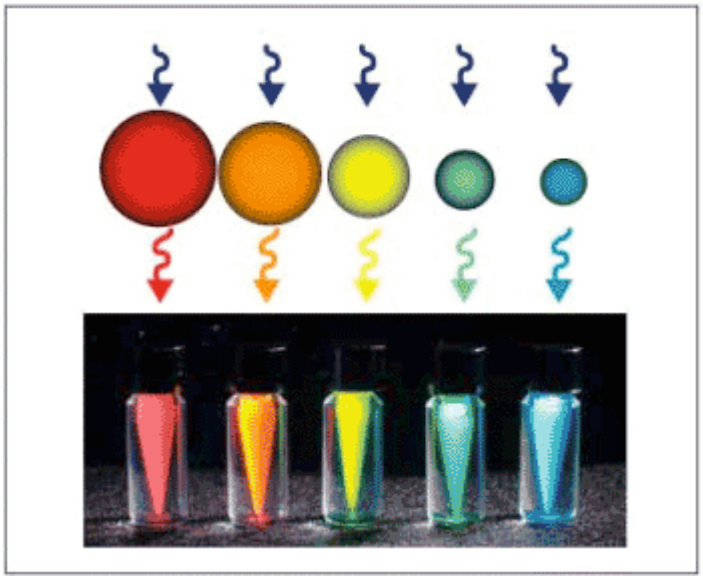
$$Q_{ads} = 4x\,\mathrm{Im}\left|\frac{m^2-1}{m^2+2}\right|^2 \propto \frac{1}{\lambda}$$

simple metals

Surface modes can be excited where Q_{ads} will become very large.

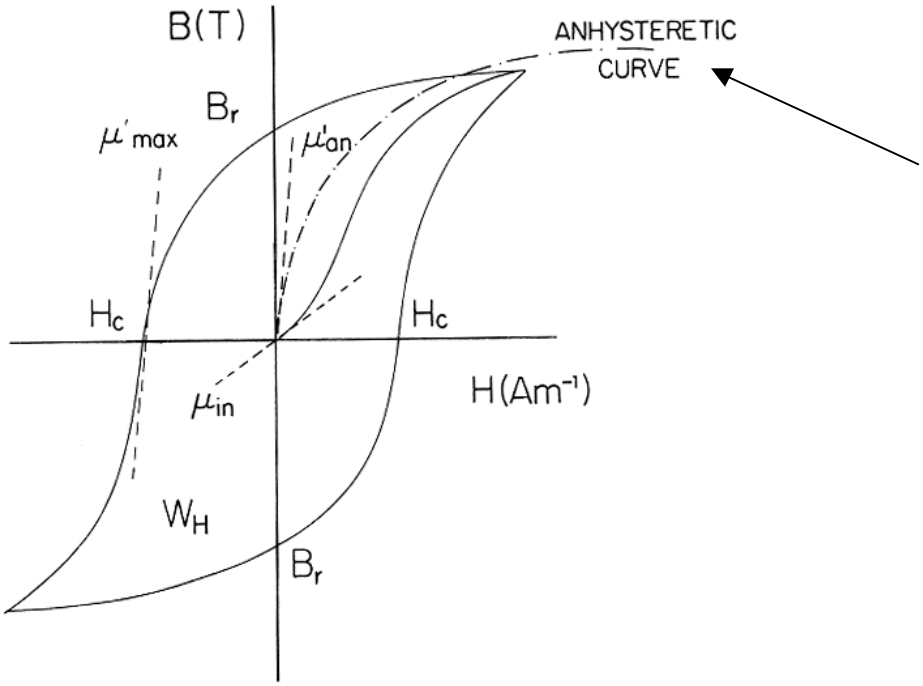
2.1 Physical Basis for Optical Properties of Nanoparticles





2.2 Physical Basis for Magnetic Properties of Nanoparticles

Ferromagnetic Material B-H Curve



Introduction to Magnetism and Magnetic Materials,
David Jiles, Chapman and Hall, 1991.

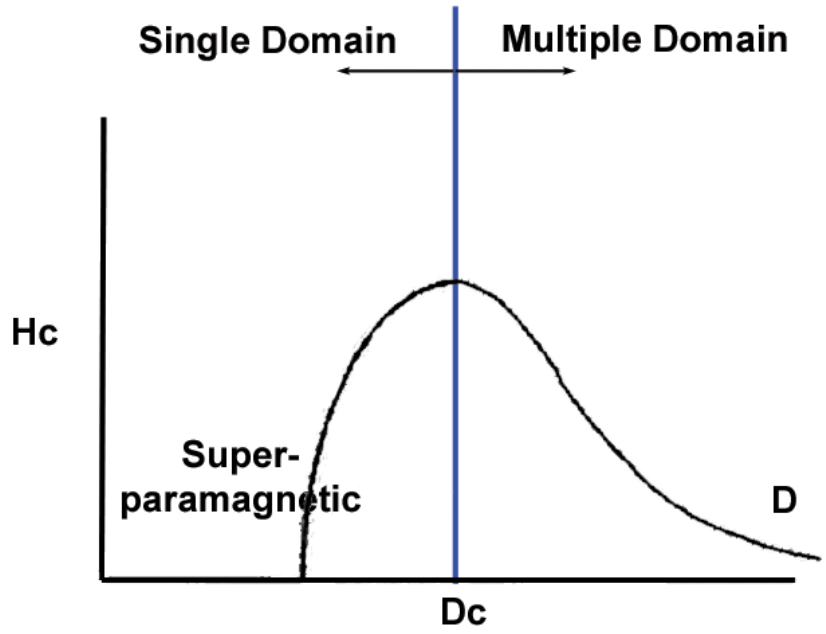
Paramagnetism

Nonhysteric B-H curve
but
Iron saturates at 1.6 T while
ferrites will normally saturate
between 200-500 mT

2.2 Physical Basis for Magnetic Properties of Nanoparticles

Ferromagnetism is based on pinning of magnetic domain walls that are 10-100 nm in scale. This can not happen in nanoparticles

Material	D_{crit} (nm)
Co	70
Fe	14
Ni	55
Fe_3O_4	128
γ-Fe_2O_3	166



The experimental criteria for superparamagnetism are: 1) the magnetization curve exhibits no hysteresis, and 2) the magnetization curves at different temperatures must superimpose in a plot of M vs H/T.

3.0 Nanoparticle Synthesis: Traditional vs. New Methods

Traditional aqueous formation of colloids depends on reduction of metal salts (Reike metals)

$$MX_n + nK \rightarrow M^* + nKX$$

Gas phase synthesis of colloidal particles has been demonstrated using vapor trapping, sputtering, and carbon arching.

New aqueous chemistries have used self-assembly and sonochemistry

3.1 Nanoparticle Synthesis: Templated Synthesis

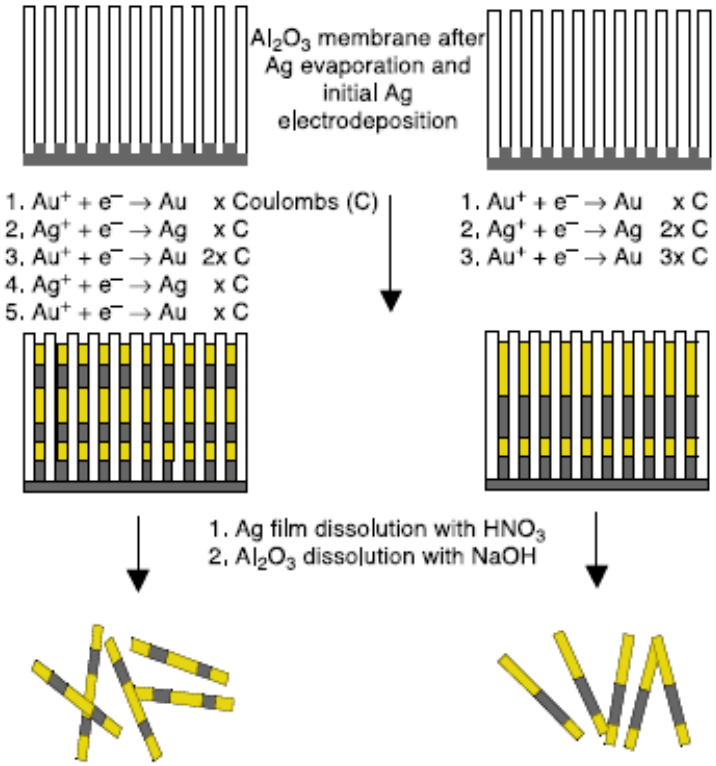
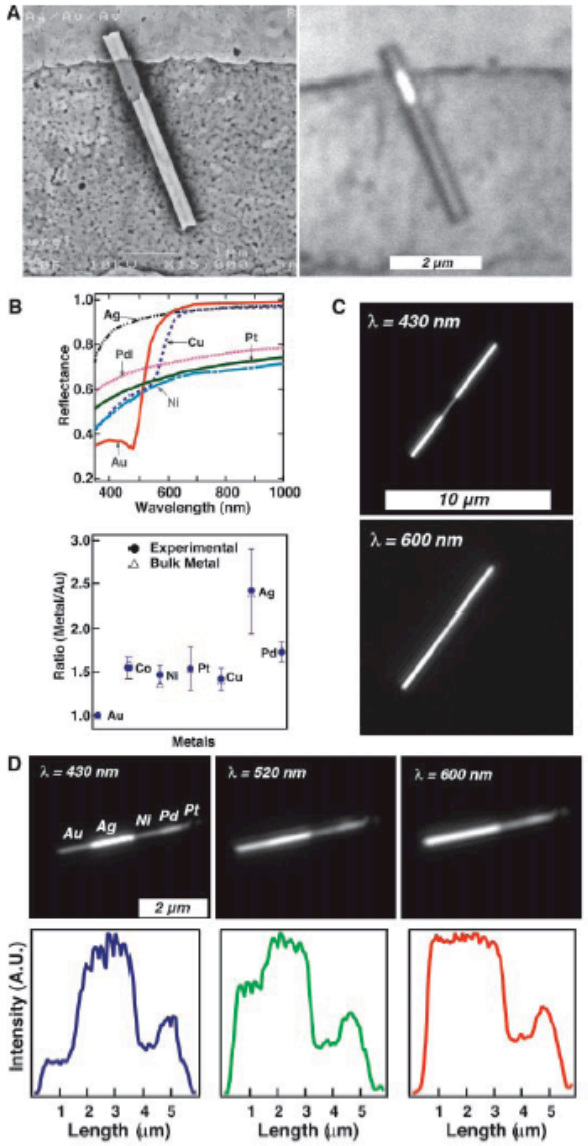


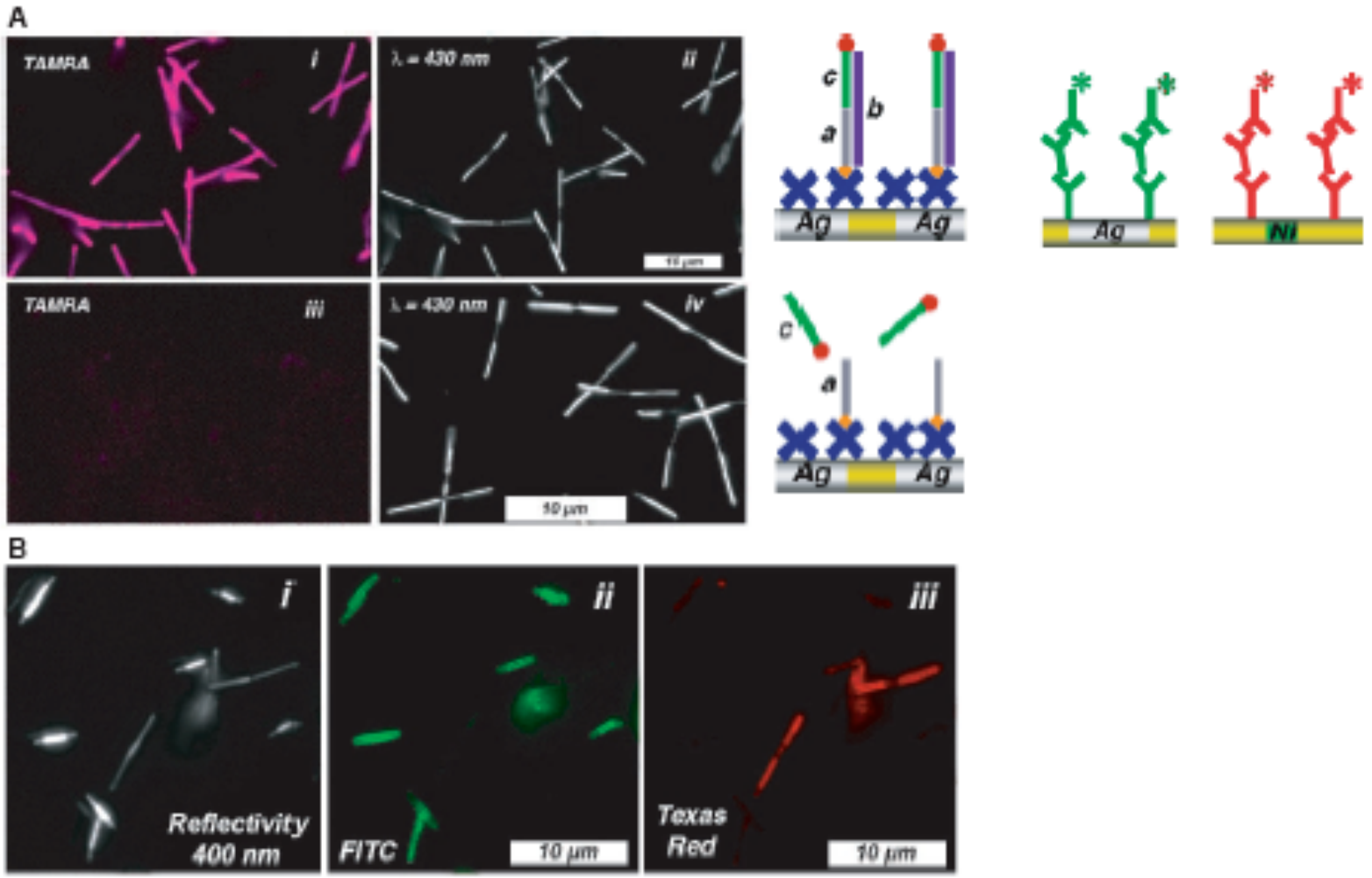
Fig. 1. Synthesis of barcoded particles.



Nicewarner-Pena, *et al*. "Submicrometer Metallic Barcodes" Science 2001, 294, 137-141.

Reiss *et al*., "Electrochemical synthesis and optical readout of striped metal rods with submicron features" J. Electroanal. Chem. 2002, 522, 95-103.

3.1 Nanoparticle Synthesis: Templated Synthesis

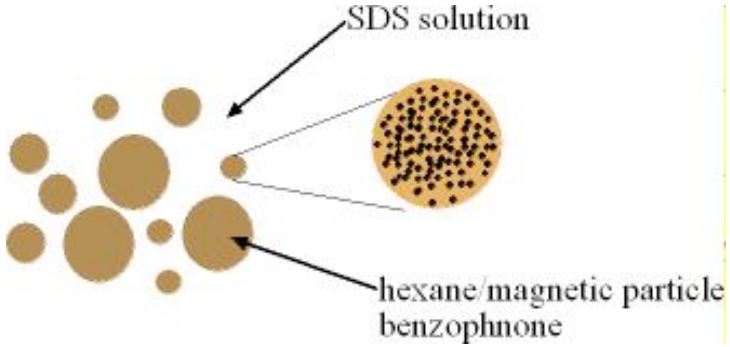


Walton *et al.*, "Particles for multiplexed analysis in solution: Detection and identification of striped metallic particles using optical microscopy." Anal. Chem. 2002, 74, 2240-2247.

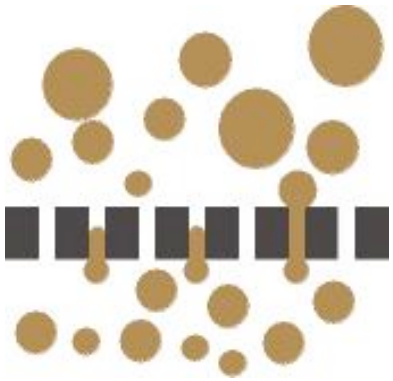
Penn *et al.* "Nanoparticles for bioanalysis." Curr Opin Chem Biol 2003, 7, 609-615.

3.3 Nanoparticle Assembly: Templated Self-Assembly

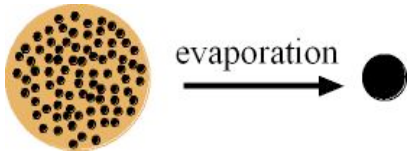
Form an emulsion from nanoparticles and initiator:



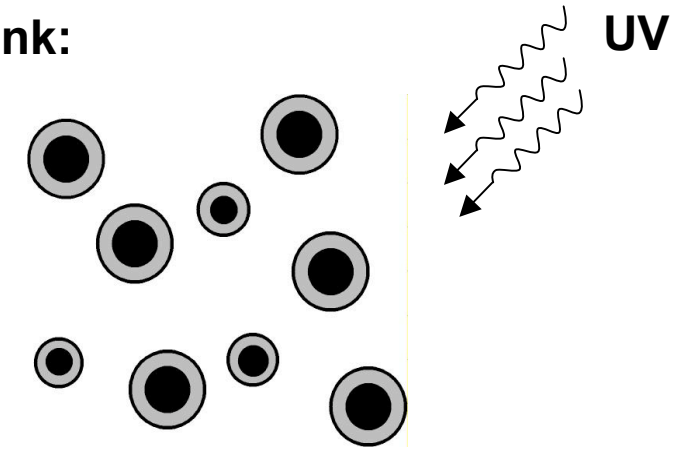
Size emulsion using extrusion:



Remove the solvent and adsorb hydrophobic monomer and surface surface active groups:

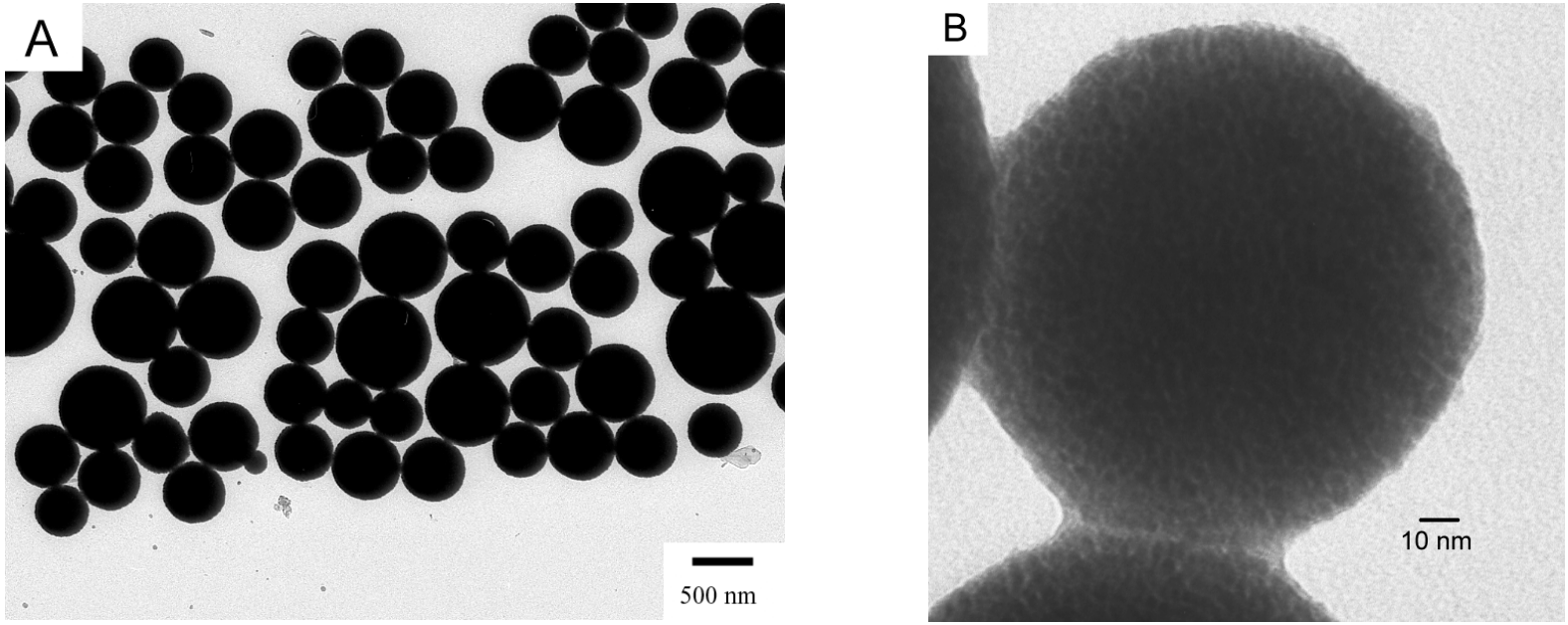


Crosslink:



3.3 Nanoparticle Assembly: Microparticle Ultrastructure

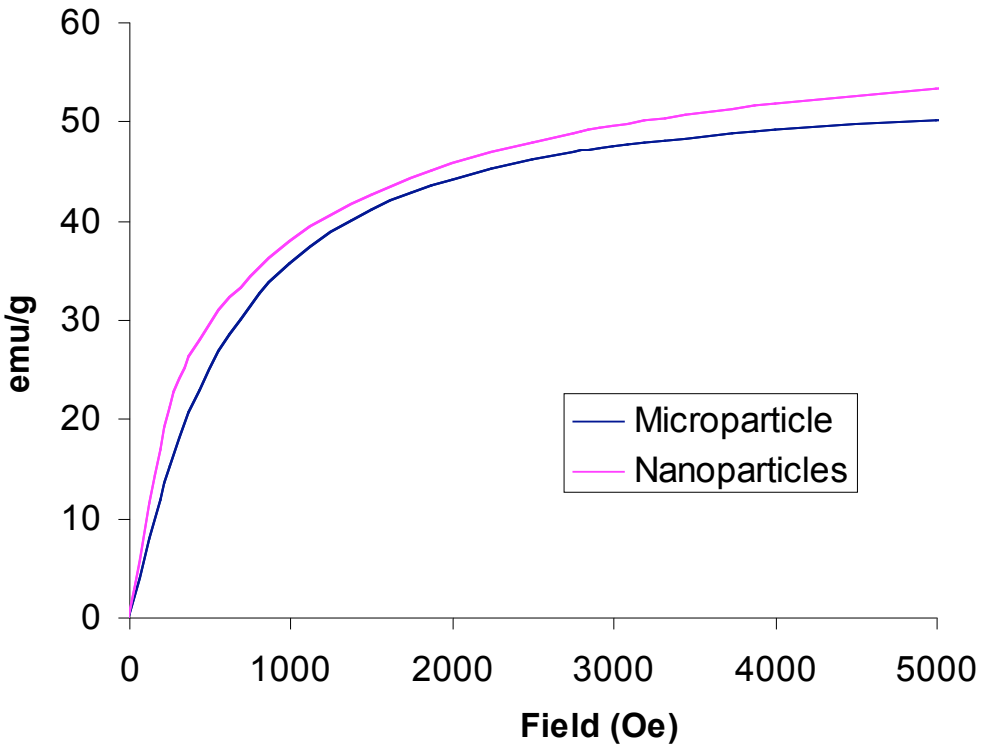
Transmission electron micrographs of the microparticles



H. Shang, et al, submitted to Langmuir 2005.

3.3 Nanoparticle Assembly: Superparamagnetic Behavior

B-H Curve for Emulsion Templated Assembly of Microparticles



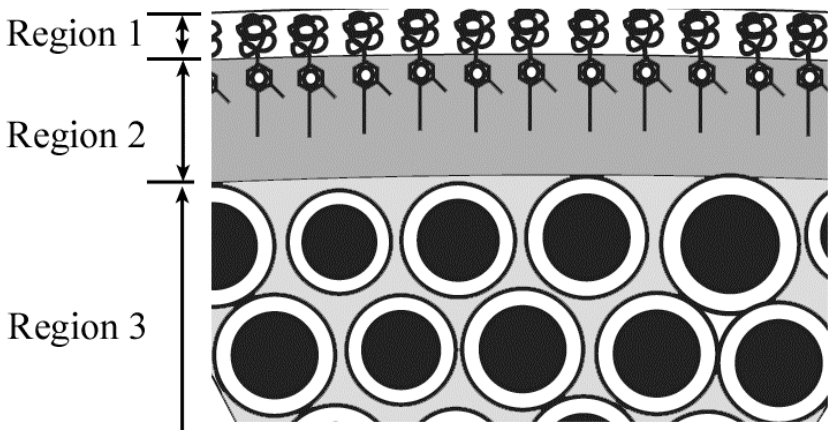
Magnetization and density measurements indicate the microparticles are > 70% by volume magnetite.

Magnetization of super paramagnetic Dynal M-280 beads is 10 emu/g and theoretical magnetization of ferromagnetic materials is 150 emu/g.

3.3 Nanoparticle Assembly: Surface Chemistry

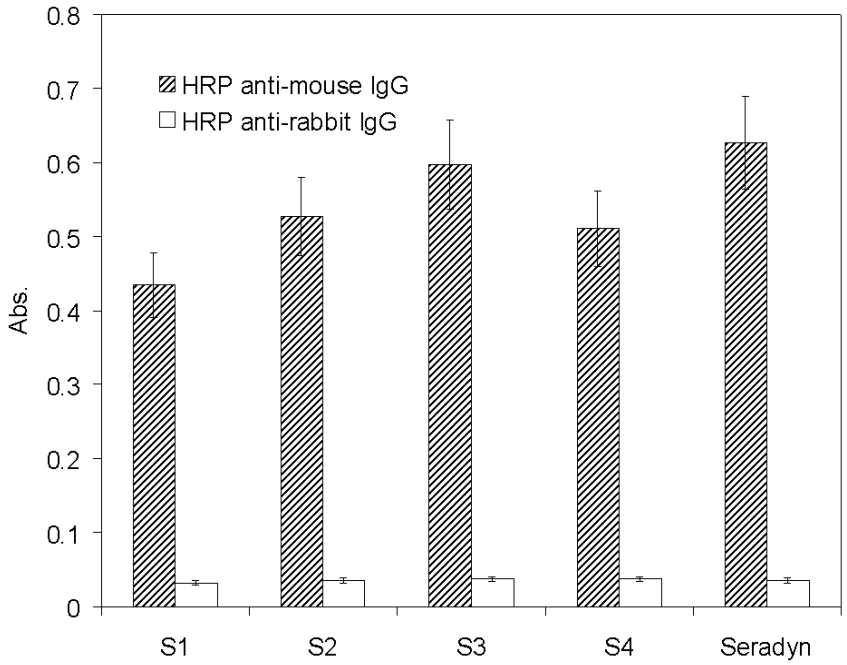
XPS Analysis of Surface Chemistry

AA:RN-10	-C*H_2CH_2 (%)	-C*HCO_2H (%)	-C*H_2CH_2O (%)	COOH/ CCO	C/Fe
4:1	26	26	22	2.3	2.5
1.5:1	25	26	23	2.2	7.5
1:1.5	27	23	25	2.0	4.9
1:4	38	16	30	1.1	2.7

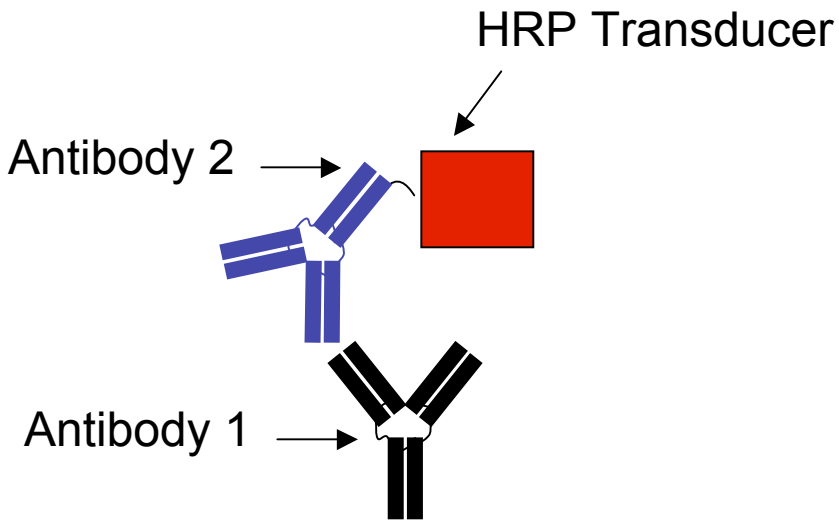


3.3 Nanoparticle Assembly: Surface Chemistry

Antibody Immobilization Produces Highly Active Materials

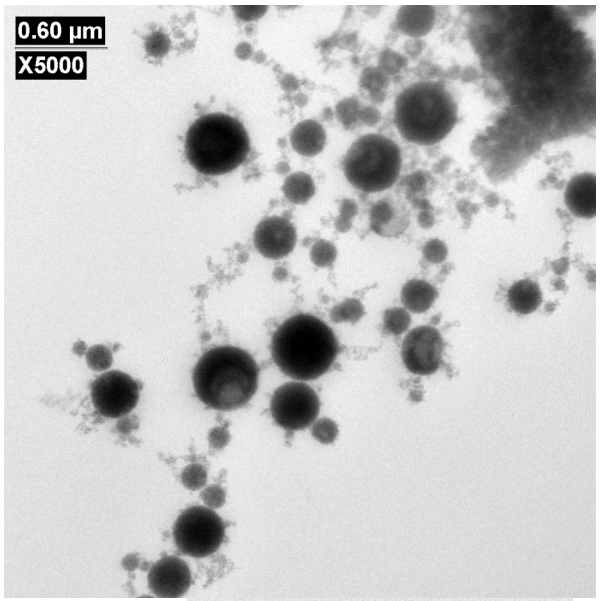


Sandwich Immunoassay

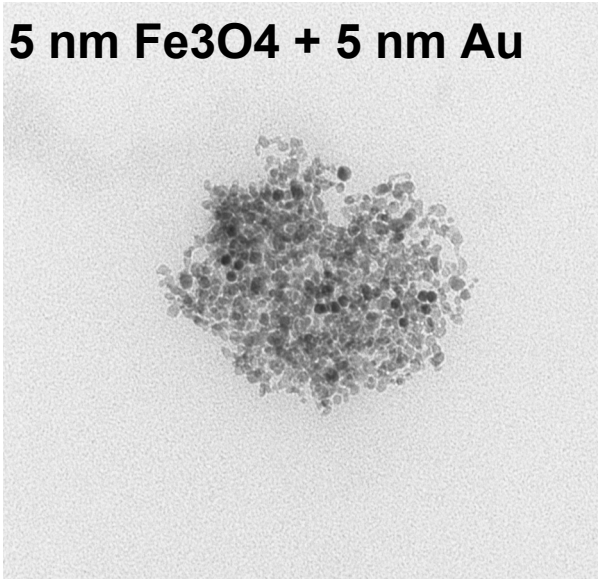


3.3 Nanoparticle Assembly: Two Particle Mixed Structures

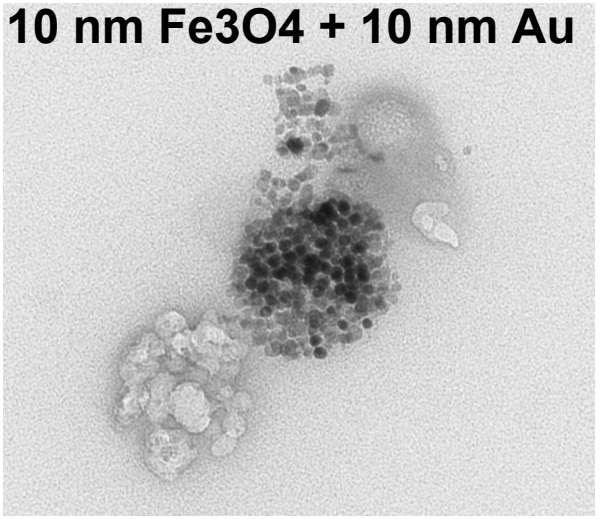
5nm Au + 20 nmFe3O4



5 nm Fe3O4 + 5 nm Au

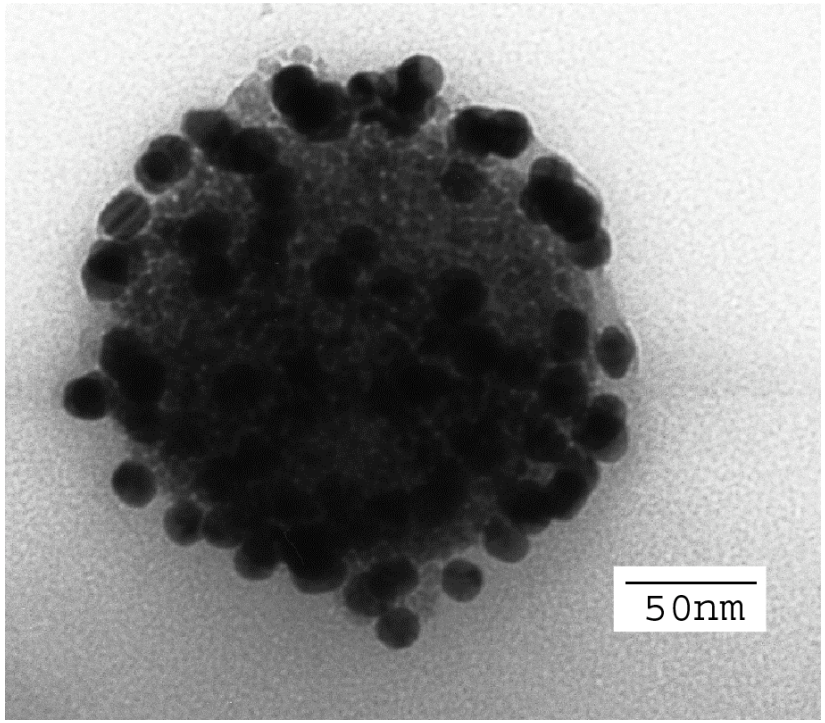


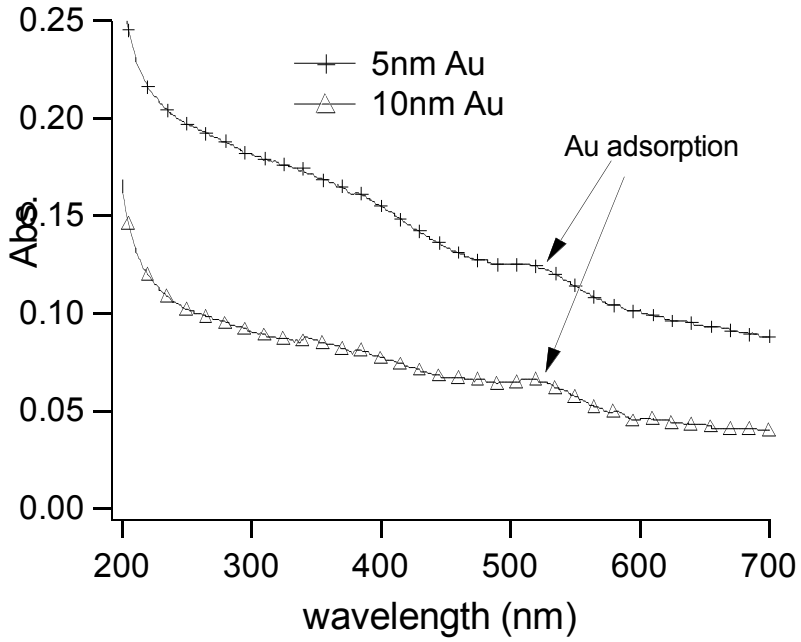
10 nm Fe3O4 + 10 nm Au



3.3 Nanoparticle Assembly: Two Particle Mixed Structures

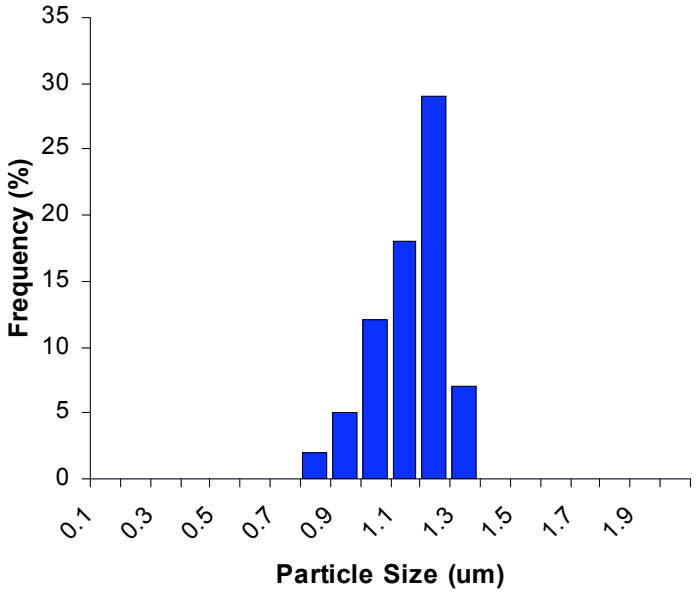
10nm Au + 20 nmFe3O4

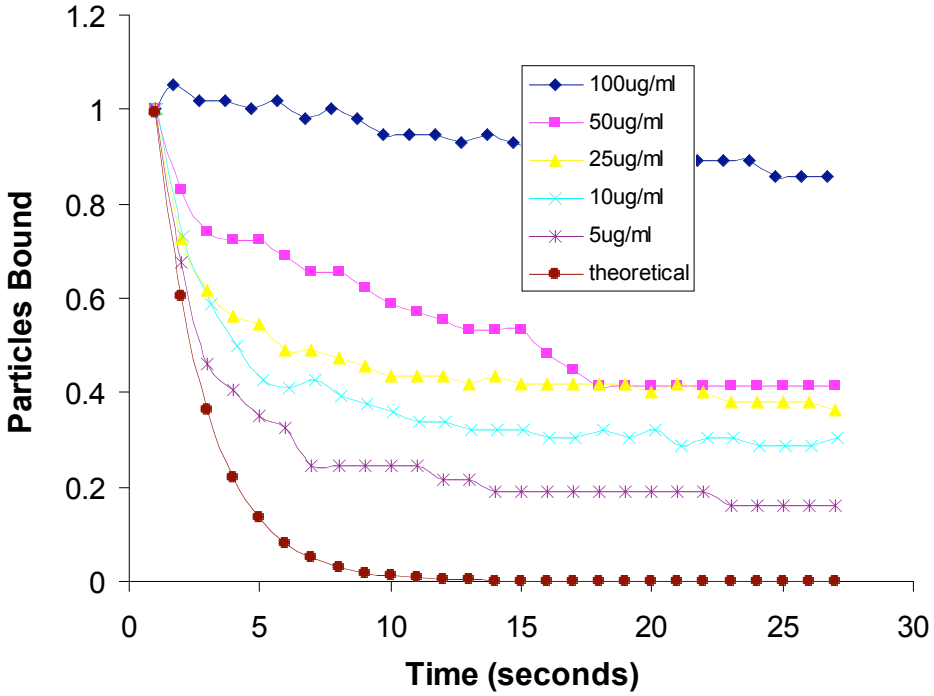




4.1 Applications: Single Molecule Measurements

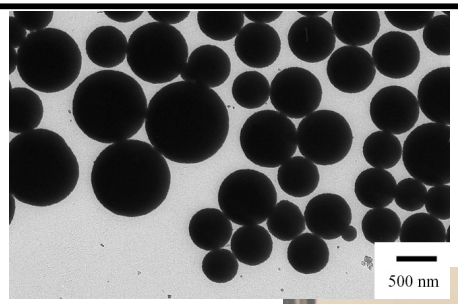
These homogeneous microparticles with high magnetic moments provide new opportunities for biophysical and analytical measurements:





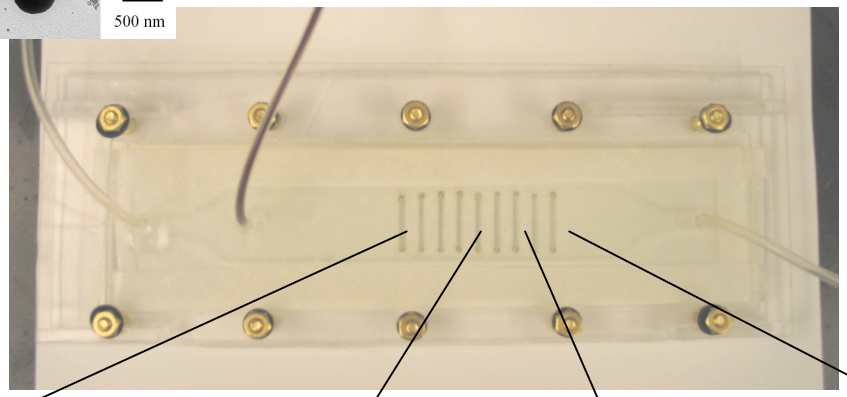
Single molecule force measurements have been made between protein A and an antibody demonstrating force differentiation for the first time.

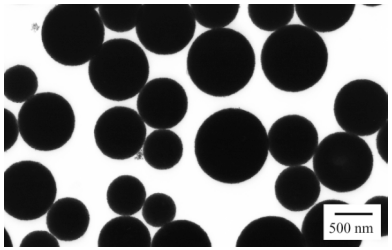
4.2 Applications: Multiplexed Magnetophoretic Assays



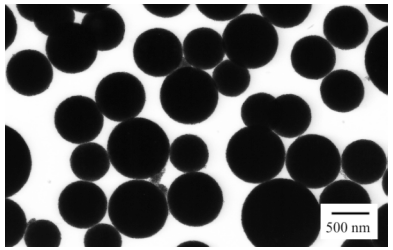
Average 0.66 um

C.V. 33%

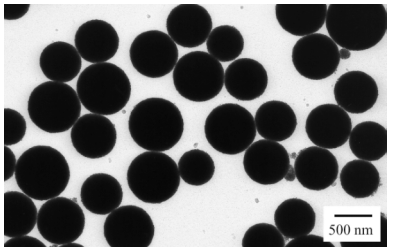




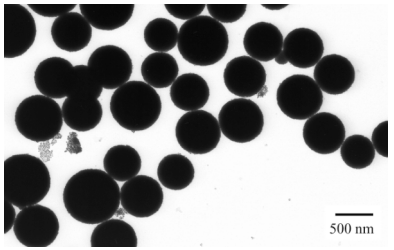
1: 0.73 um, 20%



4: 0.70 um, 19%



6: 0.64 um, 15%



8: 0.61 um, 14%

5.0 Conclusions

- Although nanoparticles are commonly used in diagnostic technologies it appears that there are excellent opportunities for new developments.
- Templated synthesis promises to allow multifunctional microparticles to be created.
- Magnetically active microparticles produced by templated synthesis promise new modes of sensing with higher sensitivities and specificities.
- Optically active microparticles promise to allow assays to be highly multiplexed allowing 100's analytes to be sensed simultaneously.

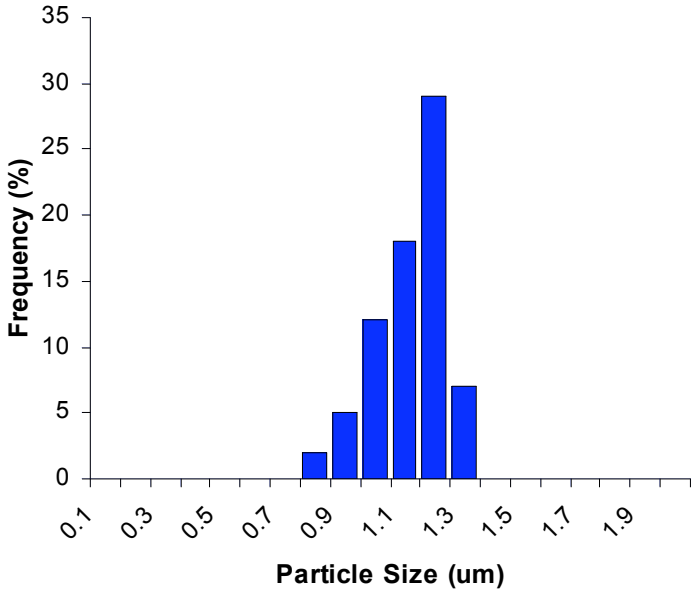
Acknowledgements

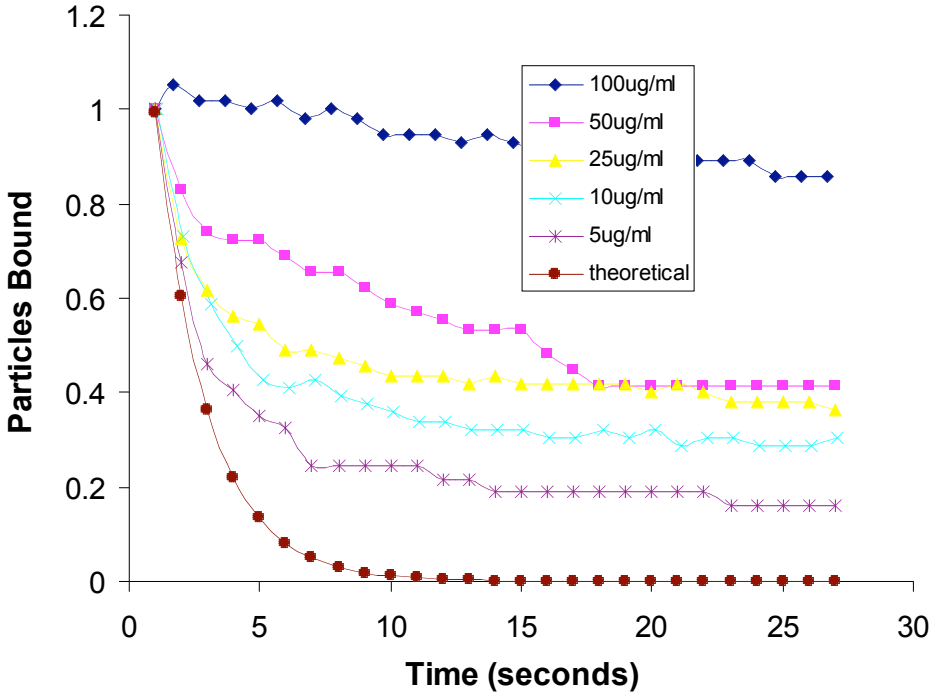
- Sarah Majetich, Department of Physics, Carnegie-Mellon.
- Rick Haasch, MicroCenter at UIUC.
- Deborah Sherman (TEM)
- Michael Benko (NASA SURI student)
- NASA INAC Center at Purdue University
- Gail Cassell, Tina Myers, and Perry Kirkham of the Infectious Diseases Division of Eli Lilly, Inc

Questions & Answers

4.1 Applications: Single Molecule Measurements

These homogeneous microparticles with high magnetic moments provide new opportunities for biophysical and analytical measurements:





Single molecule force measurements have been made between protein A and an antibody demonstrating force differentiation for the first time.