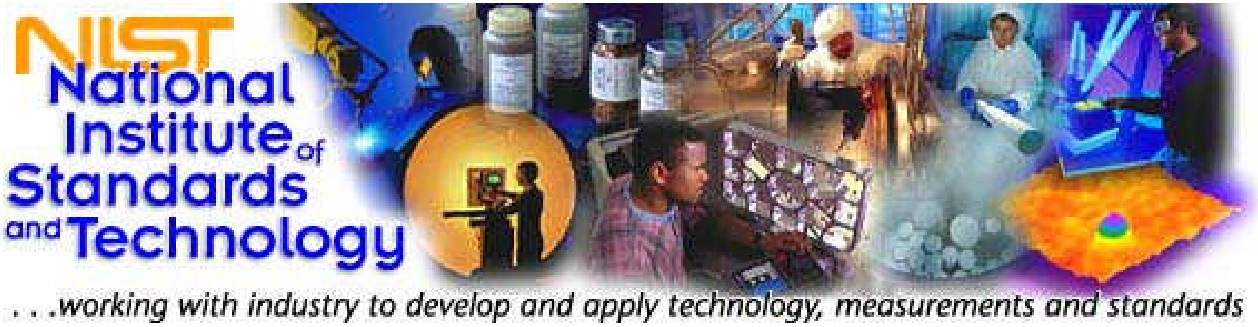

Nanoparticle Standards at NIST:
Gold Nanoparticle Reference Materials and Their Characterization

Vince Hackley
Research Chemist, Ceramics Division
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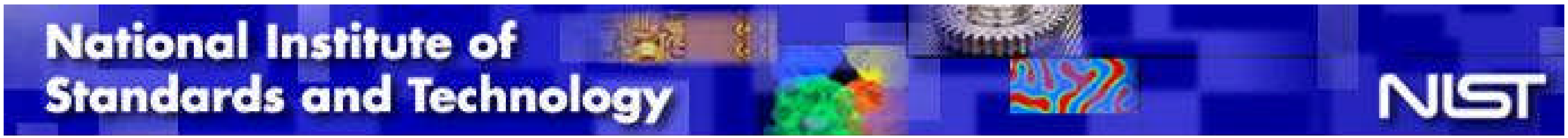

What is a Reference Material (RM)?

Reference Material:

Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

Certified Reference Material (CRM):

Reference material, characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability. Property values are *certified* as traceable to an accurate realization of the unit in which the property values are expressed.

Definitions from the International Organization for Standards (ISO)

What is a NIST (S)RM?

NIST *Reference Material* (RM):

- A CRM issued with a *Report of Investigation* that details its characterizations and provides information regarding its appropriate uses
- *Reference* values (not necessarily "NIST certified") that are the best estimates of the true values
- Associated uncertainties may reflect only measurement precision and may not include all possible sources of bias
- Reference values may be determined by e.g., a single method at NIST or data from interlaboratory studies
- Can include *information values* for which uncertainties are typically not specified

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What is a NIST (S)RM?

Table 1. Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements

		NIST Certified Value	NIST Reference Value	NIST Information Value
1.	**Certification at NIST Using a Single Primary Method with Confirmation by Other Method(s)**	Y		
2.	**Certification at NIST Using Two Independent Critically-Evaluated Methods**	Y	Y	
3.	Certification/Value-Assignment **Using One Method at NIST** and Different Methods by Outside Collaborating Laboratories	Y	Y	
4.	Value-Assignment Based On Measurements by Two or More Laboratories Using Different Methods in Collaboration with NIST		Y	Y
5.	Value-Assignment Based on a Method-Specific Protocol		Y	Y
6.	Value-Assignment Based on NIST Measurements Using a Single Method or Measurements by an Outside Collaborating Laboratory Using a Single Method		Y	Y
7.	Value-Assignment Based on Selected Data from Interlaboratory Studies		Y	Y

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Procedure for NIST (S)RM Development

Research

- Customer needs and market assessments indicate an RM is required to address barriers to innovation
- Underpinning measurement science and technology

Development

- Select material and source, define property values, select measurement methods, establish testing plan
- Develop a prototype whose reference values are not necessarily traceable

Production

- Process and package material, heterogeneity testing, generate certified, reference and information values, perform statistical analyses
- Generate COA or ROI and release RM

Development and production stages typically take 2 years

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Material Considerations for RMs

- **Source material**
  - Commercially available "off-the-shelf" material
  - "Custom-made" by NIST or an external party to NIST specifications
  - Sufficiently large, homogeneous quantity of material to produce specified number of units ("feedstock")

- **Stability of RM**
  - Ideally stable for many years; may not be possible for some materials (*e.g.,* aqueous dispersions)
  - Periodic assessment of stability with respect to property values

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Current NIST Nanoparticle (S)RMs

- **SRM 1963a** Nominal 100 nm Diameter Polystyrene Spheres
- **SRM 1964** Nominal 60 nm Diameter Polystyrene Spheres
  - Modal spherical diameter by Differential Mobility Analyzer (DMA)
  - 0.5 % mass in DI water
- **RM 8011 Gold Nanoparticles, Nominal 10 nm Diameter**
- **RM 8012 Gold Nanoparticles, Nominal 30 nm Diameter**
- **RM 8013 Gold Nanoparticles, Nominal 60 nm Diameter**
  - Mean diameter by multiple techniques
  - 0.005 % mass in water

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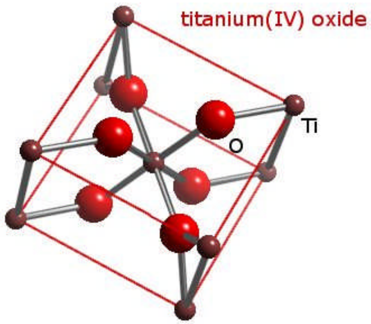
Candidate Nanoparticle RMs

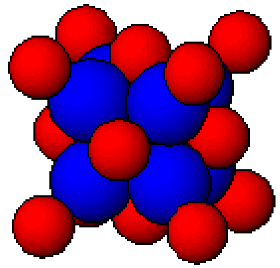
- **Titanium Dioxide (TiO_2)**
  - Cosmetics, sunscreens
  - Pigments, coatings, catalysts

Crystal structure of titanium dioxide



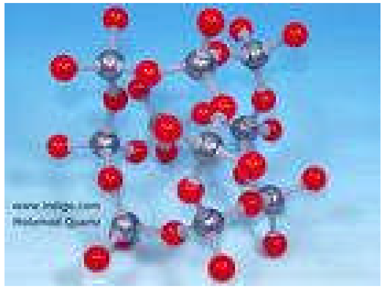
- **Silver (Ag^0)**
  - Wound dressings, surgical masks, and catheter coatings as <u>anti-microbial</u> agents
  - Soaps, toothpaste, skin creams, shampoos
  - Implantable medical devices, fabric coatings

Crystal structure of cerium dioxide



- **Cerium Oxide (CeO_2)**
  - Chemical abrasive in silicon semiconductor industry
  - Catalyst and fuel additive (UK) in automotive applications
- **Silicon Dioxide (SiO_2)**
  - Chemical abrasive
  - Food additive

Crystal structure of silicon dioxide



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Why Gold Nanoparticles (GNPs)?

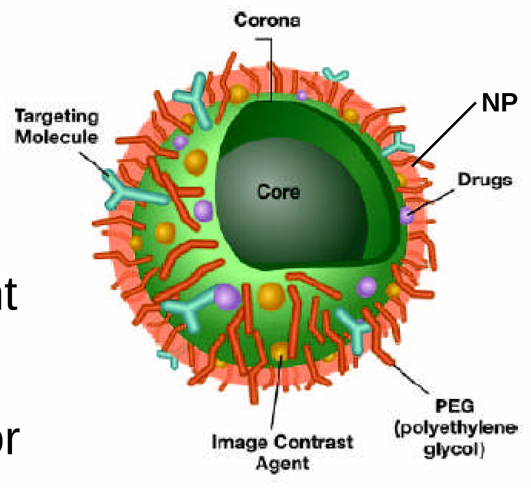
- **Gold is relatively stable and inert (exception, -SH)**
- **Gold is used in biomedical applications and is generally considered to be biocompatible**
- **The National Cancer Institute requested that NIST provide GNP reference materials:**

Gold is one “nanoparticle platform” being developed for the targeted delivery therapeutic agents to cancerous tumors

Targeting molecule binds NP to tumor

Image contrast agent “lights up” the tumor

Drug treats the tumor



S. McNeil, NCL

National Institute of Standards and Technology

Intended Uses of GNP RMs

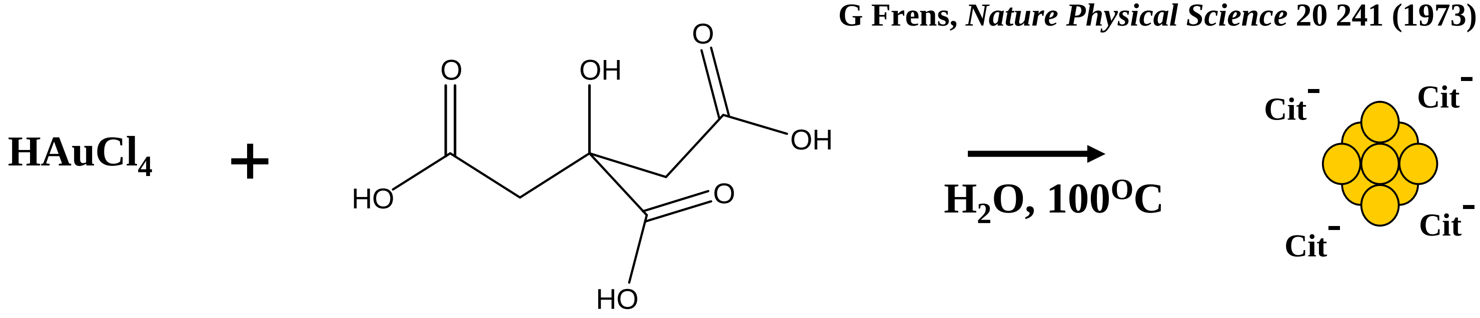
- Evaluate and qualify methodology and instrument performance related to the physical and dimensional characterization of nanoscale particles
- Develop and evaluate *in vitro* and *in vivo* assays designed to assess the biological response (*e.g.,* cytotoxicity, hemolysis) of nanomaterials
- Develop and evaluate assays to assess the impact of nanomaterials in the environment and the risks to human health
- Facilitate interlaboratory comparisons and benchmarking

Federal Agencies interested in using gold NP RMs: *National Cancer Institute (NCI), Food and Drug Administration (FDA), National Toxicology Program (NTP), Environmental Protection Agency (EPA), National Institute for Occupational Safety and Health (NIOSH)*

GNP RM Material and Packaging

- Citrate-reduced gold in aqueous solution (0.01% $HAuCl_4$)

G Frens, *Nature Physical Science* 20 241 (1973)



- Produced by BBInternational to NIST specifications in 8 liter batches for 3 nominal sizes:

  RM8011 – 10 nm

  RM8012 – 30 nm

  RM8013 – 60 nm

- Gold solutions homogenized, hermetically sealed in 5 mL glass ampoules under argon, gamma-irradiated to ensure sterility.

- Each RM “package” contains two 5 mL ampoules.

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Reference Values for GNP RMs

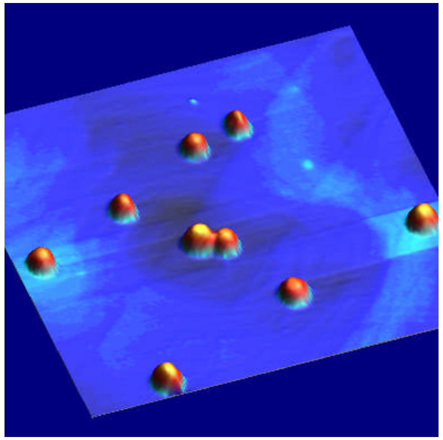
Reference Value Definition

A value best estimate of the true value provided on the NIST Report of Investigation, where all known or suspected sources of bias have not been fully investigated by NIST.

Gold NP RMs: nominal 10, 30, and 60 nm

Mean NP diameter as measured by:

- Atomic Force Microscopy (AFM)
- Scanning Electron Microscopy (SEM)
- Transmission Electron Microscopy (TEM)
- Dynamic Light Scattering (DLS)
- Electrospray-Differential Mobility Analysis (ES-DMA)
- Small-Angle X-ray Scattering (SAXS)



60 nm gold NPs

Note: *NPs are roughly spherical with few agglomerates*

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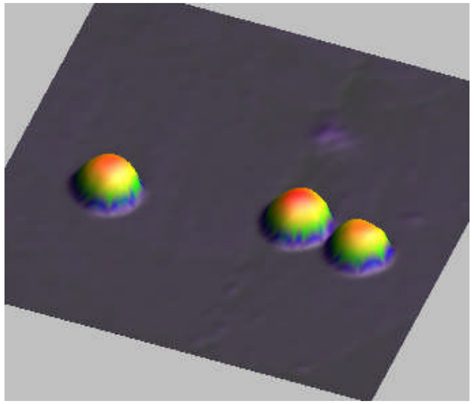
Mean GNP Diameter by AFM



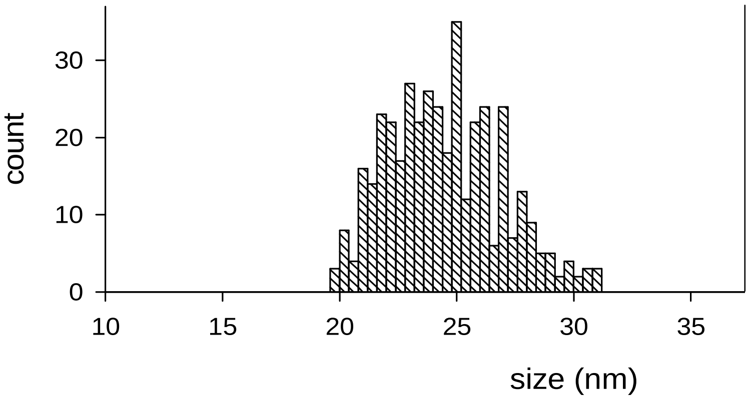
AFM image of 30 nm gold NPs

Sample Preparation and Form:

- Gold solution centrifuged and diluted to remove citrate salt
- Resulting solution dried onto gold (111)-mica substrate

Measurements:

- Measure maximum height of the NP with reference to the baseline substrate in tapping mode
- Traceable to a known wavelength of light via a 68.9 nm silicon step height transfer artifact
- Over 300 NPs measured for each size



AFM data on particle size distributions for 30 nm gold NPs

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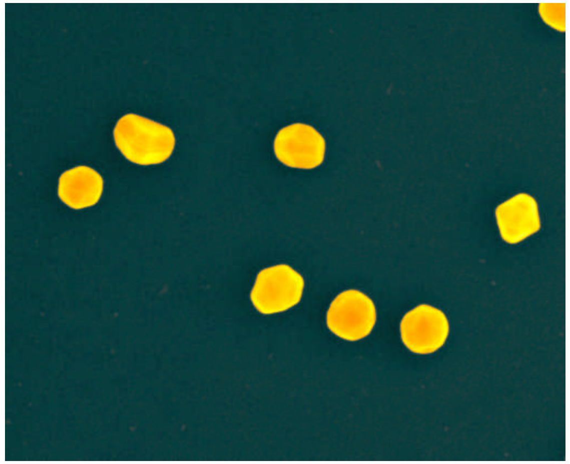
Mean GNP Diameter by SEM

Sample Preparation and Form:

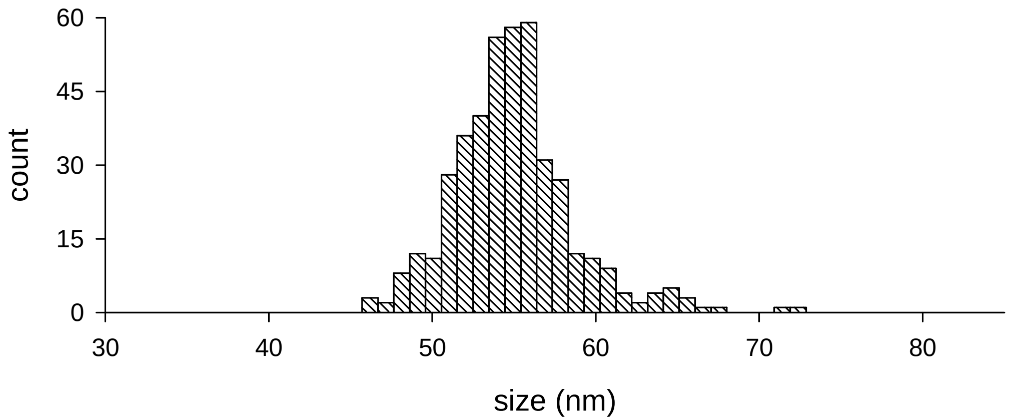
- Gold solution deposited electrostatically onto an aminosilane-modified Si substrate with a thin native oxide layer
- Residual solution removed by rinsing



SEM image of 60 nm gold NPs

Measurements:

- Measure particle area (pixels) and convert to mean particle diameter assuming a spherical shape
- Traceable to a calibrated AFM using a lattice transfer artifact
- 100s of NPs measured for each size



SEM data on particle size distributions for 60 nm gold NPs

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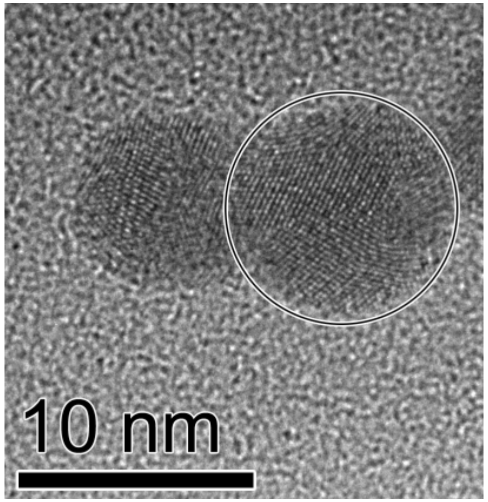
Mean GNP Diameter by TEM

Sample Preparation and Form:

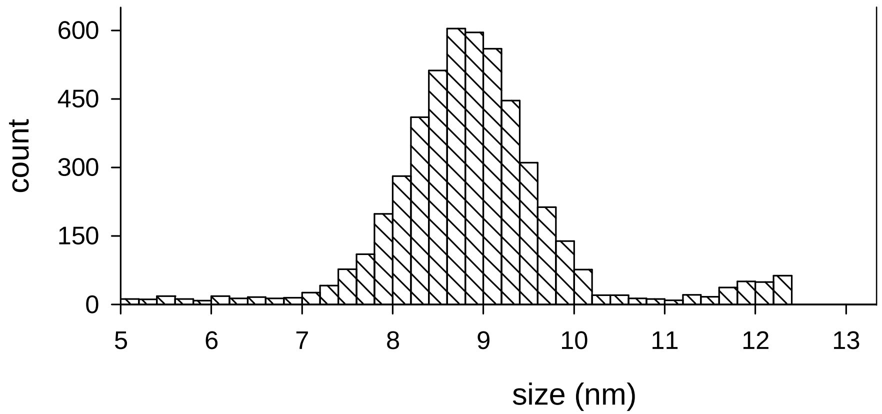
- Gold solution deposited electrostatically onto an aminosilane-modified SiO_2-coated grid
- Residual solution removed by rinsing

Measurements:

- Measures projected images of NPs
- Measure particle area (pixels) and convert to mean particle diameter assuming a spherical shape
- Instrument calibrated using negatively stained catalase crystals
- 1000s of NPs measured for each size



TEM image of 10 nm gold NPs



TEM data on particle size distributions for 10 nm gold NPs

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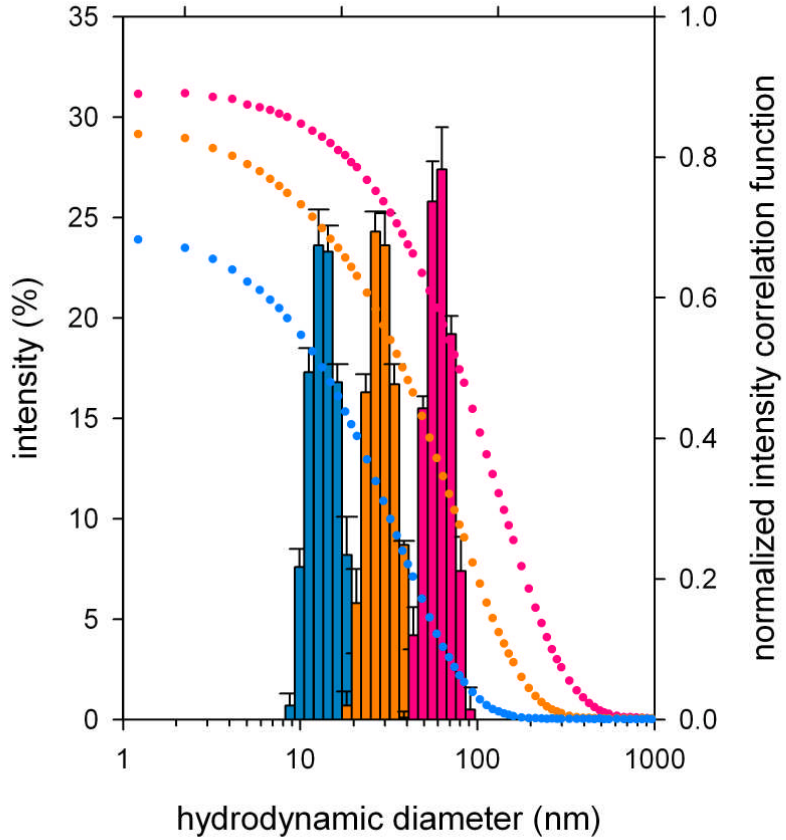
Mean GNP Diameter by DLS

Sample Preparation and Form:

- Native gold diluted into 2 mol/m^3 NaCl solution
- Passed through 0.1 µm or 0.45 µm membrane filter

Measurements:

- Measure intensity fluctuations of scattered light in solution to determine z-average ensemble particle diameter
- Instrument performance qualified against NIST SRM 1964 (nominal 60 nm diameter polystyrene spheres)
- 40 individual measurements averaged for each size



DLS data on particle size distributions for 10 nm, 30 nm, and 60 nm gold NPs

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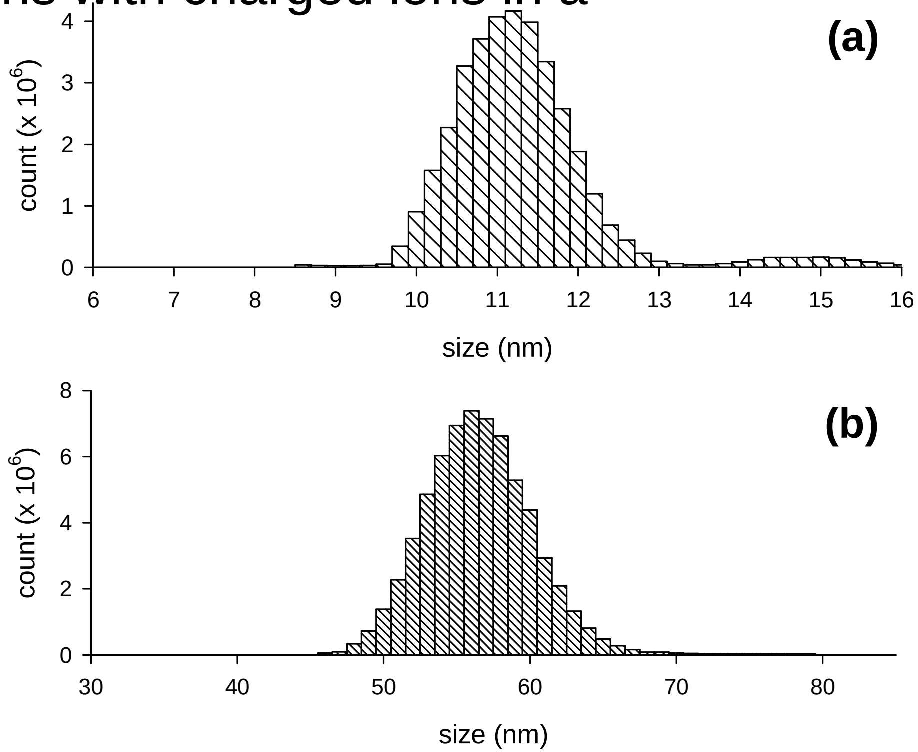
Mean GNP Diameter by ES-DMA

Preparation of Sample and Form:

- Gold solution modified with electrolyte and conveyed to gas phase using electrospray ionization
- Droplets become charged by collisions with charged ions in a neutralizing chamber

Measurements:

- Vary voltage on negatively-biased electrode and detect with condensation particle counter
- Convert DMA voltage to average equivalent spherical diameters
- 10^6+ particles counted for each size



DMA data on particle size distributions for (a) 10 nm and (b) 60 nm gold NPs

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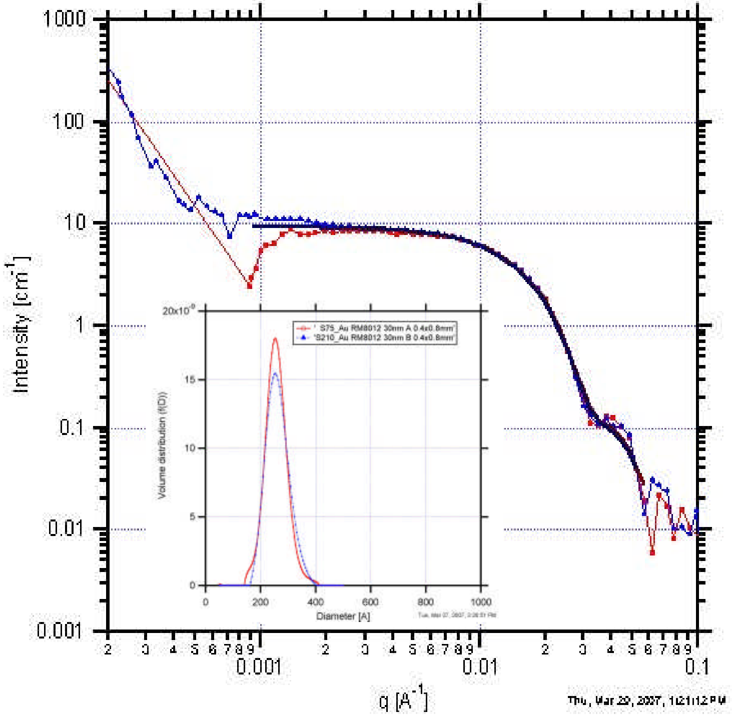
Mean GNP Diameter by SAXS

Preparation of Sample and Form:

- Native gold solution loaded into static cells
- Native gold solution loaded into a capillary flow cell to analyze material under flowing conditions



Ultra-SAXS data on particle size distributions for 30 nm gold NPs

Measurements:

- Pass a well-collimated beam of hard x-rays through a sample and measure the scattered intensity at small solid angles to the incident
- Performed in laboratory and synchrotron-based SAXS instruments
- Fit data to hard sphere model to obtain ensemble average NP diameter
- Four samples measured in static load cells for each size
- One sample measured under flow conditions for each size

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Summary of Mean GNP Diameter Results

Technique	Analyte Form	Nominal 10 nm	Nominal 30 nm	Nominal 60 nm
AFM	dry, deposited on substrate	8.5 ± 0.3	24.9 ± 1.1	55.4 ± 0.3
SEM	dry, deposited on substrate	9.9 ± 0.1	26.9 ± 0.1	54.9 ± 0.4
TEM	dry, deposited on substrate	8.9 ± 0.1	27.6 ± 2.1	56.0 ± 0.5
ES-DMA	dry, aerosol	11.3 ± 0.1	28.4 ± 1.1	56.3 ± 1.5
DLS (a)	diluted liquid suspension	13.5 ± 0.1	28.6 ± 0.9	56.6 ± 1.4
DLS (b)	diluted liquid suspension	--	26.5 ± 3.6	55.3 ± 8.3
SAXS	native liquid suspension	9.1 ± 1.8	24.9 ± 1.2	53.2 ± 5.3

DLS results: (a): 173° scattering angle; (b) 90° scattering angle

The reported expanded uncertainty reflects a 95% uncertainty interval, and is calculated according to ISO and NIST guides.

Different techniques measure different aspects of NP dimensions under different conditions, so it is expected that the results will not be identical. The RM user should refer to the value specified for their technique.

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Information Values in Gold NP RMs

Information Value Definition

A value best estimate of the true value provided on the NIST Report of Investigation; insufficient information is available to assess adequately the uncertainty associated with the values or a limited number of analyses were performed.

Gold RM Reports of Investigation:

- Chemical & electrochemical properties:
  - Gold (Au) and sodium (Na) concentrations by ICP-OES
  - Citrate and chloride (Cl) concentrations by ion chromotography
  - pH
  - Electrolytic conductivity
  - Zeta potential (electrophoretic mobility) by Doppler velocimetry
- UV-Vis optical absorbance spectrum
- Chromatographic separation trace by field flow fractionation
- Sterility and endotoxin assessment

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ICP-OES: inductively-coupled plasma optical emission spectrometry

Summary of Information Values

Chemical and Electrochemical Properties

Measurement	Units	Nominal 10 nm NPs	Nominal 30 nm NPs	Nominal 60 nm NPs
Au mass fraction	$\mu g\ g^{-1}$	51.56 ± 0.23	48.17 ± 0.33	51.86 ± 0.64
Cl^- ion mass fraction	$\mu g\ g^{-1}$	35.0 ± 2.3	32.1 ± 1.2	36.3 ± 0.6
Citrate ion mass fraction	$\mu g\ g^{-1}$	1.7 ± 0.2	< 0.02	< 0.02
Na mass fraction	$\mu g\ g^{-1}$	95	--	--
pH	---	7.19 ± 0.33	7.04 ± 0.32	7.30 ± 0.32
Electrolytic conductivity, κ	$\mu S\ cm^{-1}$	417.9 ± 7.2	208.4 ± 6.5	241.6 ± 6.5
Zeta potential (Smol.Eq.)	mV	--	-33.6 ± 6.9	-37.6 ± 3.0
Electrophoretic mobility	$\mu m\ cm\ V^{-1}\ s^{-1}$	--	-2.38 ± 0.49	-2.67 ± 0.21

Informational values are considered to be of interest to the RM user.

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Report of Investigation

Document that accompanies the RM and which details the RM's characterization and provides information regarding its appropriate uses.

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Report of Investigation

Reference Material 8012

Gold Nanoparticles, Nominal 30 nm Diameter

This Reference Material (RM) is intended primarily to evaluate and qualify methodology and/or instrument performance related to the physical/dimensional characterization of nanoscale particles used in pre-clinical biomedical research. The RM may also be useful in the development and evaluation of in vitro assays designed to assess the biological response (e.g., cytotoxity, hemolysis) of nanomaterials, and for use in interlaboratory test comparisons. RM 8012 consists of nominally 5 mL of citrate-stabilized Au nanoparticles in an aqueous suspension, supplied in hermetically sealed pre-scored glass ampoules sterilized by gamma irradiation. A unit consists of two 5 mL ampoules. The suspension contains primary particles (monomers) and a small percentage of clusters of primary particles.

Expiration of Material: The reference values for RM 8012 are valid, within the measurement uncertainties specified, until **31 December 2012**, provided the RM is handled in accordance with the instructions given in this report (see "Instructions for Use"). However, the size distribution may be altered and the RM invalidated if the material is contaminated or handled improperly.

Contents of Report of Investigation

- Introductory Statement and Intended Use
- Expiration of Material
- Maintenance of RM Value Assignment (stability evaluation)
- Contribution Acknowledgements (participants, funding)
- Reference Value Statement and Table (value + uncertainty)
- Information Value Statement and Data (footnoted methods)
- Handling and Storage
- Instructions for Use & Cautions to User
- Material Source and Processing
- Heterogeneity Assessment - UV-Vis, DLS and [Au]
- Value Assignment and Uncertainty Analysis
- Methods for Reference Value Measurements (instrument, sample prep procedures, measurement & analysis procedures)
- References

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RMs Available Now for Sale

Order RMs on line, $239.00 per unit
https://srmors.nist.gov/orderingSRMs.cfm

Reference Material 8011
Gold Nanoparticles, Nominal 10 nm Diameter

Reference Material 8012
Gold Nanoparticles, Nominal 30 nm Diameter

Reference Material 8013
Gold Nanoparticles, Nominal 60 nm Diameter

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Statistical Design and Analysis: A.I. Avilés (Statistical Engineering Division)

Packaging and Issuance of the RMs: Measurement Services Division

Additional Technical Aspects and Coordination: R.F. Cook, W.K. Haller, and D.L. Kaiser (Ceramics Division)

Develop. & Production Costs: subsidized by National Cancer Institute

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