Gold Nanoparticles

Marie-Christine Daniel and Didier Astruc*

Chem. Rev. **2004,** *104,* 293-346

Scanometric DNA Array Detection with Au Nanoparticle Probes

Detection of Cancer cells at very early stage.

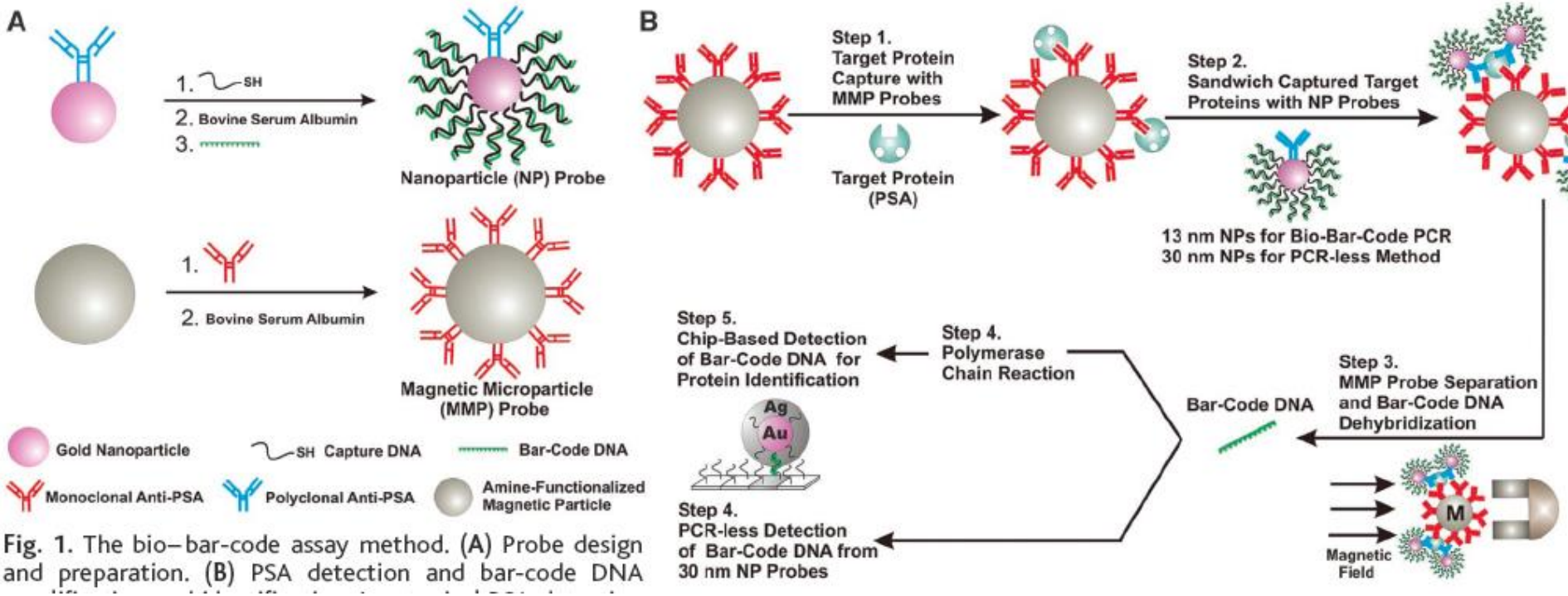
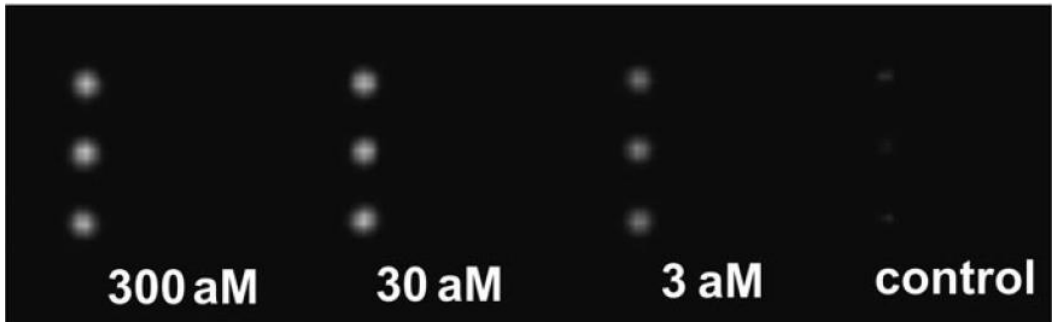


Fig. 1. The bio–bar-code assay method. (A) Probe design and preparation. (B) PSA detection and bar-code DNA



J.-M. Nam, C. S. Thaxton, C. A. Mirkin *Science* **2003**, 301, 1884.

T. Andrew Taton,1,2 Chad A. Mirkin,1,2* Robert L. Letsinger1* *Science* **2000**, 289, 1757.

서울대 화학부-BK화학분자공학사업단-기초과학연구원 나노입자 연구단 석학강연

SNU Chemistry Department-BK Chemistry and Molecular Engineering Division-IBS Center for Nanoparticle Research Distinguished Lecture

· 4:30 pm, December 3, 2014 · 서울대학교 상산수리과학관 대형강의실(129동101호)
Sangsan Mathematical Science Building, SNU(Lecture Room 101, Building 129)

Programmable Atom Equivalents from Nucleic-Acid Modified Nanostructures: Constructing a New "Table of Elements"

Professor Chad A. Mirkin

Department of Chemistry and International Institute for Nanotechnology Northwestern University, USA

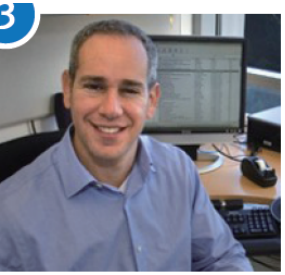
The crystallographic parameters of atomic and ionic solids are fixed by the size and coordination number of their elemental building blocks, thus restricting the types of structures that can be formed. We have demonstrated that these limitations can be overcome using spherical nucleic acid (SNA) nanoparticle conjugates as “artificial atoms” in superlattice assemblies. These three-dimensional conjugates consist of densely functionalized, highly oriented nucleic acids covalently attached to the surface of inorganic nanoparticles.The strength and length of the programmable DNA “bonds” between these structures can be adjusted by varying DNA sequence and length, and the properties of the “atoms” can be adjusted by varying nanoparticle size, shape, and composition.
We have developed design rules for this assembly process, analogous to Pauling’s Rules for ionic solids but ultimately more powerful. These rules can be used as a guide for the rational construction of functional nanoparticle-based materials for plasmonic, photonic, and catalytic applications.

Dr. Chad A. Mirkin is the Director of the International Institute for Nanotechnology, the George B. Rathmann Professor of Chemistry, Professor of Chemical and Biological Engineering, Professor of Biomedical Engineering, Professor of Materials Science & Engineering, and Professor of Medicine. He is a chemist and a world renowned nanoscience expert, who has authored over 590 manuscripts, and inventor on over 930 patent applications worldwide (251 issued).

Dr. Mirkin has been recognized for his accomplishments with over 100 national and international awards. These include the Linus Pauling Medal, the $500,000 Lemelson-MIT Prize, the Raymond and Beverly Sackler Prize in the Physical Sciences, the Feynman Prize in Nanotechnology, an Honorary Degree from Nanyang Technological Univ. Singapore, the Lee Kuan Yew Distinguished Visitor to Singapore, and the ACS Award for Creative Invention. He is a Member of the President's Council of Advisors on Science & Technology (PCAST, Obama Administration), and one of only 15 scientists, engineers, and medical doctors to be elected to all three US National Academies (the Institute of Medicine, the Natl. Academy of Sciences, and the Natl. Academy of Engineering). He is also a Fellow of the American Academy of Arts and Sciences. He is the founding editor of the journal Small and the founder of three companies, Nanosphere, Inc., AuraSense, LLC, and AuraSense Therapeutics, LLC. Dr. Mirkin holds a B.S. degree from Dickinson College and a Ph.D. degree from Penn State. He was an NSF Postdoc at MIT prior to becoming a Professor at Northwestern in 1991.

·문의(contact): 서울대학교 화학부 남좌민 교수 (jmnam@snu.ac.kr)

Seminar during our lecture time on Thursday, December 04



Dr. Bruce Cohen (LBNL Molecular Foundry Research Staff, Biological Nanostructures)

주제 | Lanthanide–Doped Inorganic Nanocrystals that Make Exceptional Single–Molecule Imaging Probes

약력 | UC Berkley, Ph. D., Department of Chemistry
UC San Francisco, Postdoctoral Fellow
Howard Hughes Medical Institute, Postdoctoral Fellow
LBNL Molecular Foundry, Research Staff

About Research Proposal

- **Due date: December 16, 6 pm**
- **Submit to Dr. Ryoo at SUN-JONG RYOO sjryoo@snu.ac.kr**
- **< 3 pages in pdf format**
- **Title, your name, Thesis advisor, Affiliation,**

  Background, main idea, references

The Impact of Nanoscience on Heterogeneous Catalysis

Alexis T. Bell, *Science* **2003**, 299, 1688.

[10] a) M. Haruta, S. Tsubota, T. Kobayashi, H. Kageyama, M. J. Genet, B. Delmon, *J. Catal.* **1993**, *144*, 175–192; b) M. Haruta, M. Daté, *Appl. Catal. A* **2001**, *222*, 427–437.

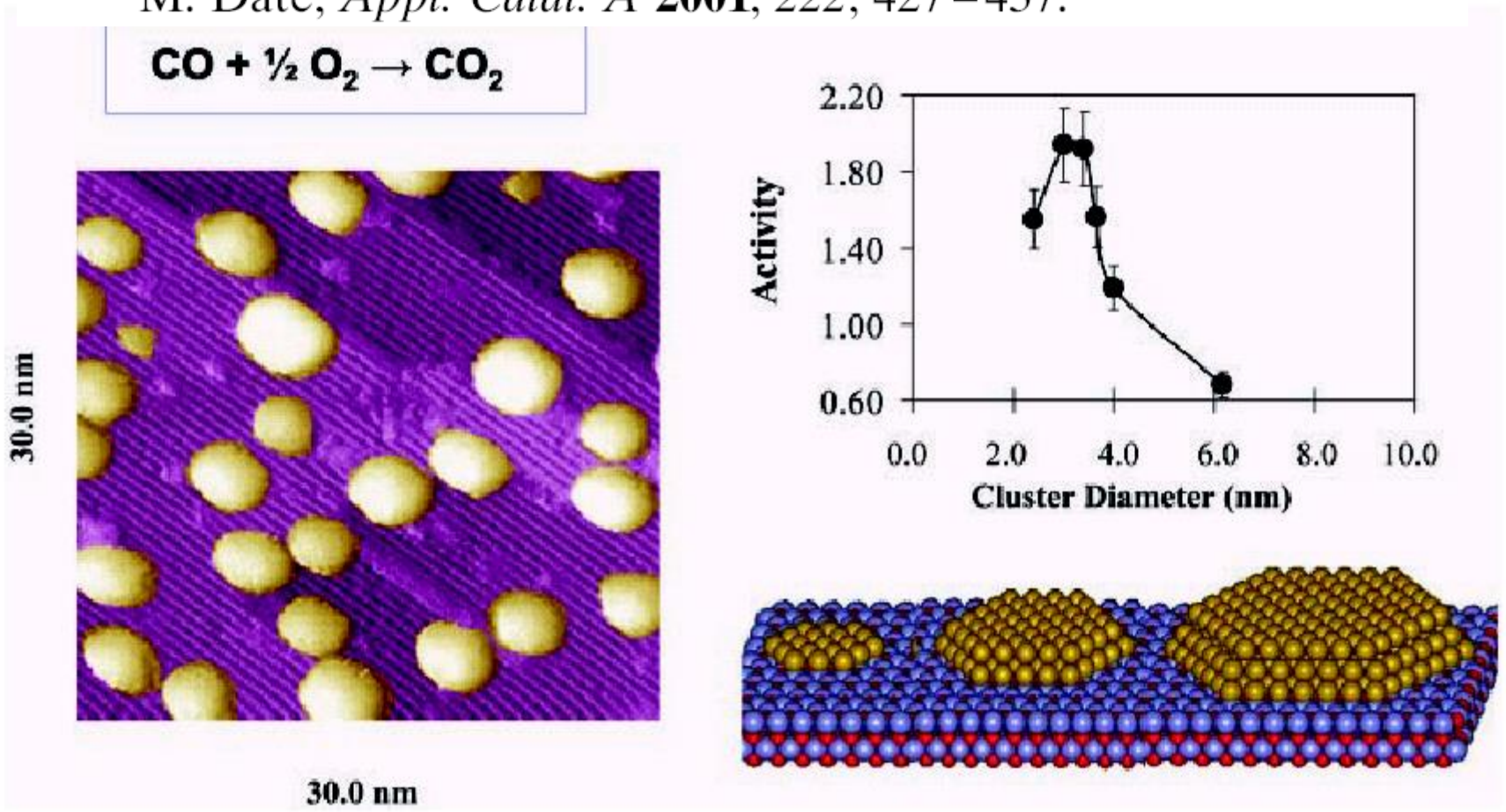
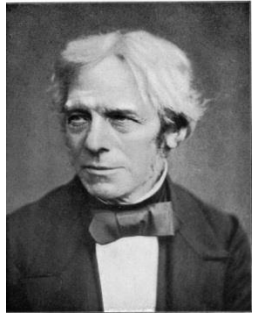
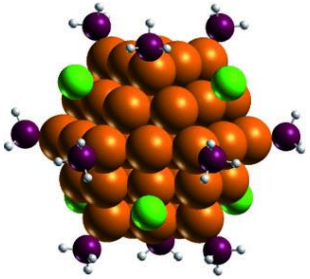


Fig. 2. Effects of particle size on the activity of titania-supported Au for the oxidation of CO (*5*).



Historical background

- In 1857, Faraday reported the formation of deep red solutions of colloidal gold by reduction of anaqueous solution of chloroaurate ($AuCl_4^-$) using phosphorus in CS_2 (a two-phase system).
- The most popular method for a long time has been that using citrate reduction of HAuCl4 in water, which was introduced by Turkevitch in 1951.



- Schmid: Au_{55} cluster (1.4 nm) synthesis
- Brust: Two phase synthesis of Au nanoparticles
- Mirkin: Biosensor applications

The Lycurgus Cup 4th century, Late Roman

green in reflected light; ruby red in transmitted light

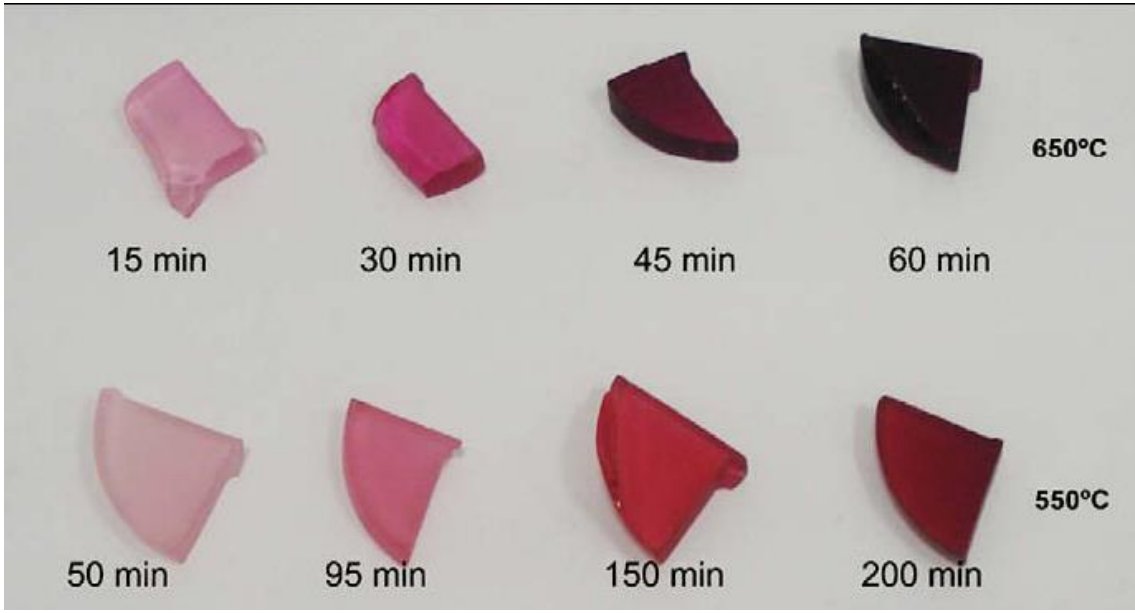
http://www.britishmuseum.org/explore/highlights/highlight_objects/pe_mla/t/the_lycurgus_cup.aspx

Gold nanoparticles in ancient and contemporary ruby glass, Andreia Ruivo, et al., *Journal of Cultural Heritage* 9 (**2008**) e134-e137.

Gold ruby glass was made by irradiating a soda-lime-silicate glass with ca. 0.2 weight % of gold with gamma rays and further heating instead of using a reducing agent such as stannous oxide.

Red-purple glass vase, 17th century, Monastery of Santa Clara-a-Velha, Coimbra.

Different colours were obtained by controlling the temperature and heating times.



Soda-lime-silicate glass heated at 550 C and 650 C during different times.

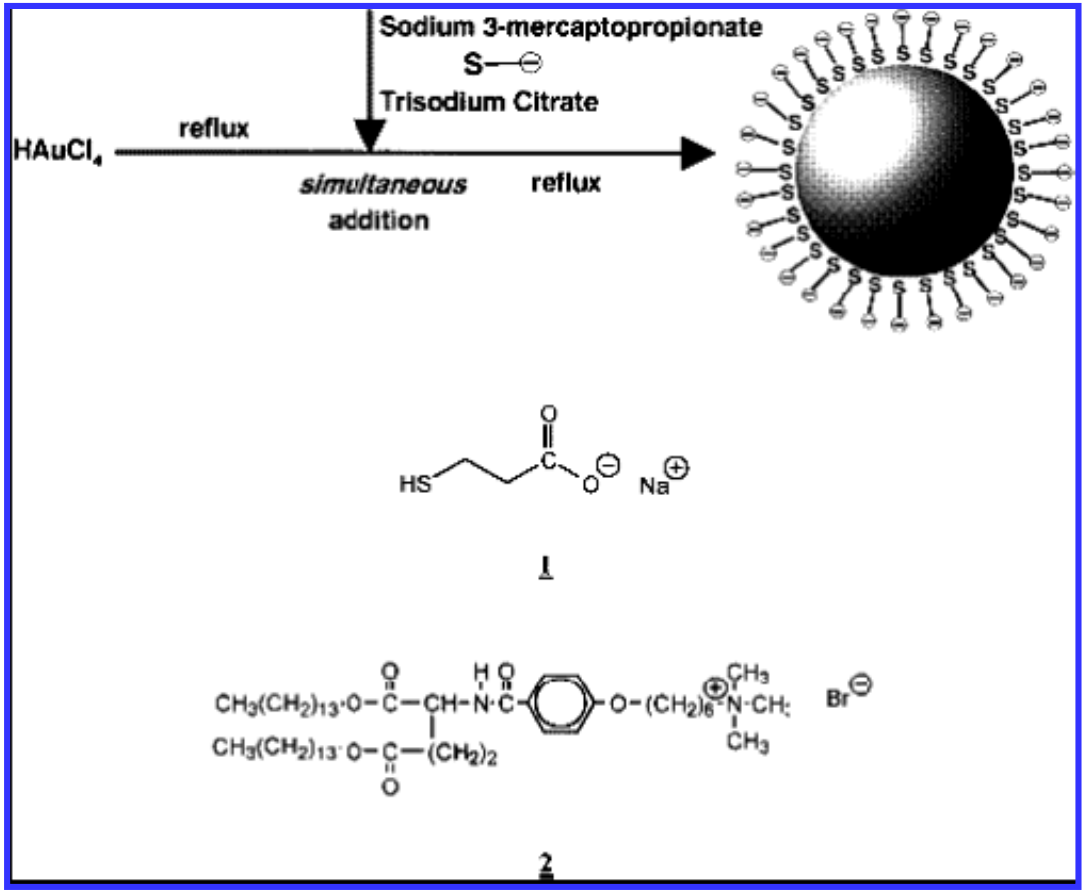
Au clusters

- The pioneering work by Schmid and co-workers on well-defined phosphine-stabilized gold clusters
- The number of atoms in these gold clusters is based on the dense packing of atoms taken as spheres, each atom being surrounded by 12 nearest neighbors. Thus, the smallest cluster contains 13 atoms, and the following layers contain $10n^2 + 2$ atoms, n being the layer number.
- For instance, the second layer contains 42 atoms, which leads to a total of 55 atoms for a gold cluster, and the compound $[Au_{55}(PPh_3)_{12}Cl_6]$ has been well characterized by Schmid's group.
- Recently, spectroscopic data have revealed discrete energy level spacings of 170 meV that can be attributed to the Au_{55} core.
- Larger clusters containing, respectively, 147, 309, 561, 923,
- 1415, or 2057 (n = 3~8) atoms have been isolated.

Schmid, G. Large Clusters and Colloids. *Chem. Rev.* **1992**, 92, 1709-1727.

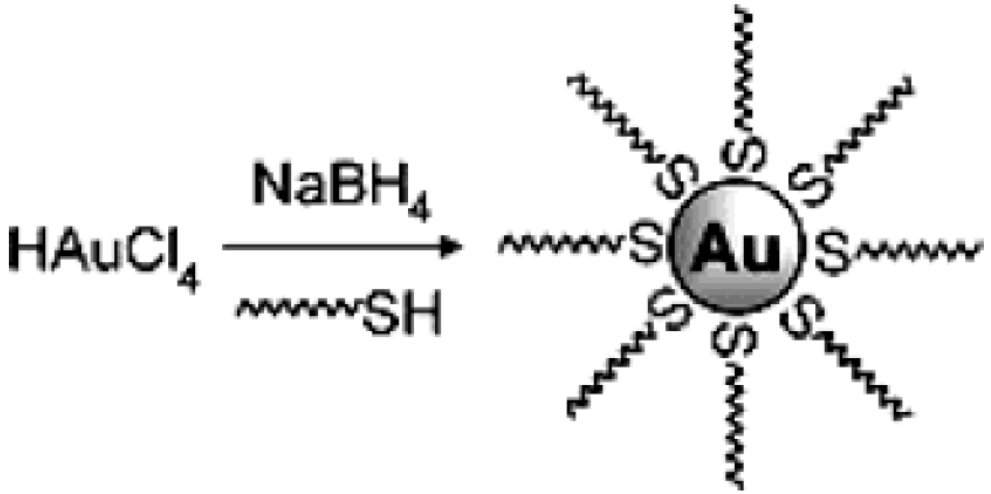
- The stabilization of AuNPs with alkanethiols was first reported in 1993 by Mulvaney and Giersig, who showed the possibility of using thiols of different chain lengths.

Giersig, M.; Mulvaney, P. Preparation of ordered colloid monolayers by electrophoretic deposition. *Langmuir* **1993**, *9*, 3408



Synthesis of Thiol-Derivatized Gold Nanoparticles in a Two-phase Liquid-Liquid System.

Brust, M. *J. Chem. Soc., Chem. Commun.* **1994**, 801-802.



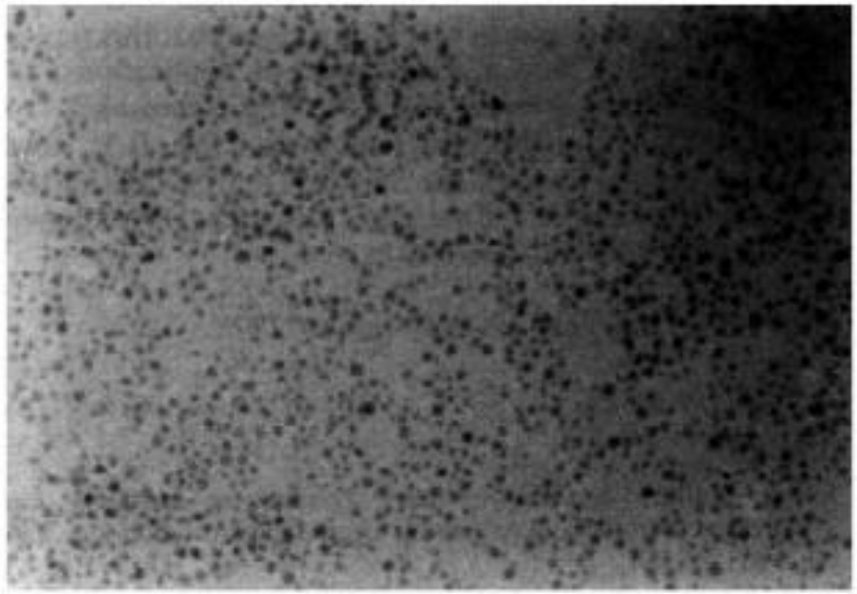
$$AuCl_4^-(aq) + N(C_8H_{17})_4^+(C_6H_5Me) \rightarrow N(C_8H_{17})_4^+AuCl_4^-(C_6H_5Me)$$

$$m AuCl_4^-(C_6H_5Me) + n C_{12}H_{25}SH(C_6H_5Me) + 3m\, e^- \rightarrow 4m\, Cl^-(aq) + [Au_m(C_{12}H_{25}SH)_n](C_6H_5Me)$$

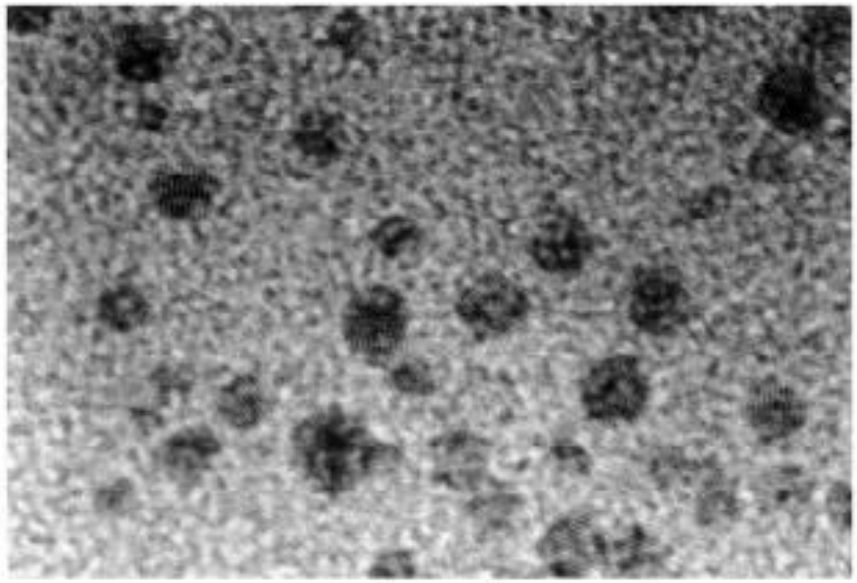
• Brust reported a so-called two-phase (or biphasic) method for synthesizing relatively uniform-sized 1-3 nm gold nanoparticles from the reduction of a gold salt in a toluene solution.

• In this process, Hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$) dissolved in water was transferred into a toluene solution containing alkanethiol using tetraoctylammonium bromide (TOAB, $(octyl)_4N^+Br^-$) as a phase transfer reagent.

• The subsequent reduction was performed by adding $NaBH_4$ to the toluene solution with vigorous stirring. This biphasic method has been used extensively for synthesizing gold nanoparticles as well as nanoparticles of other noble metals.



(*b*)



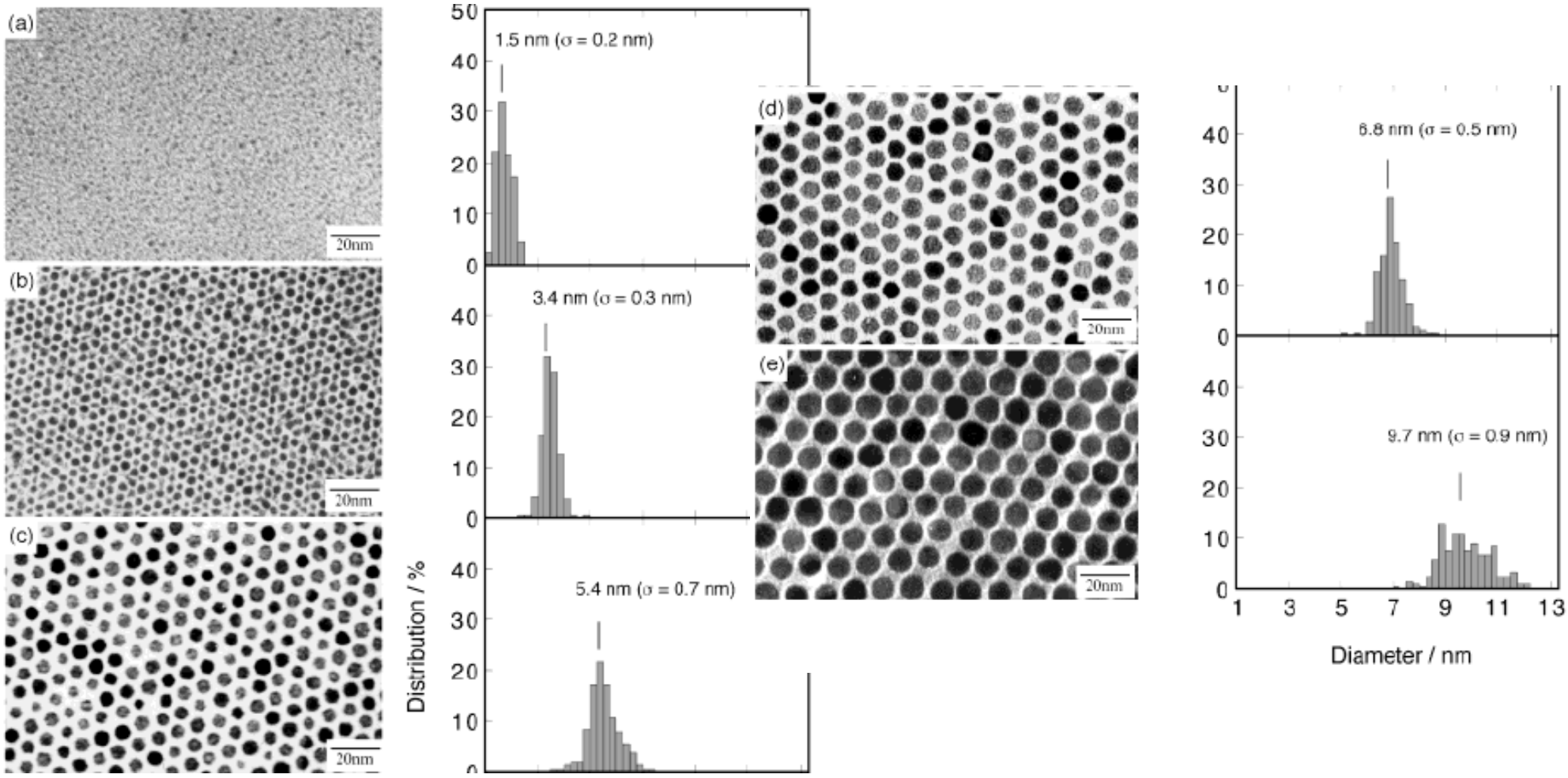
Size Evolution of Alkanethiol-Protected Gold Nanoparticles by Heat Treatment in Solid state

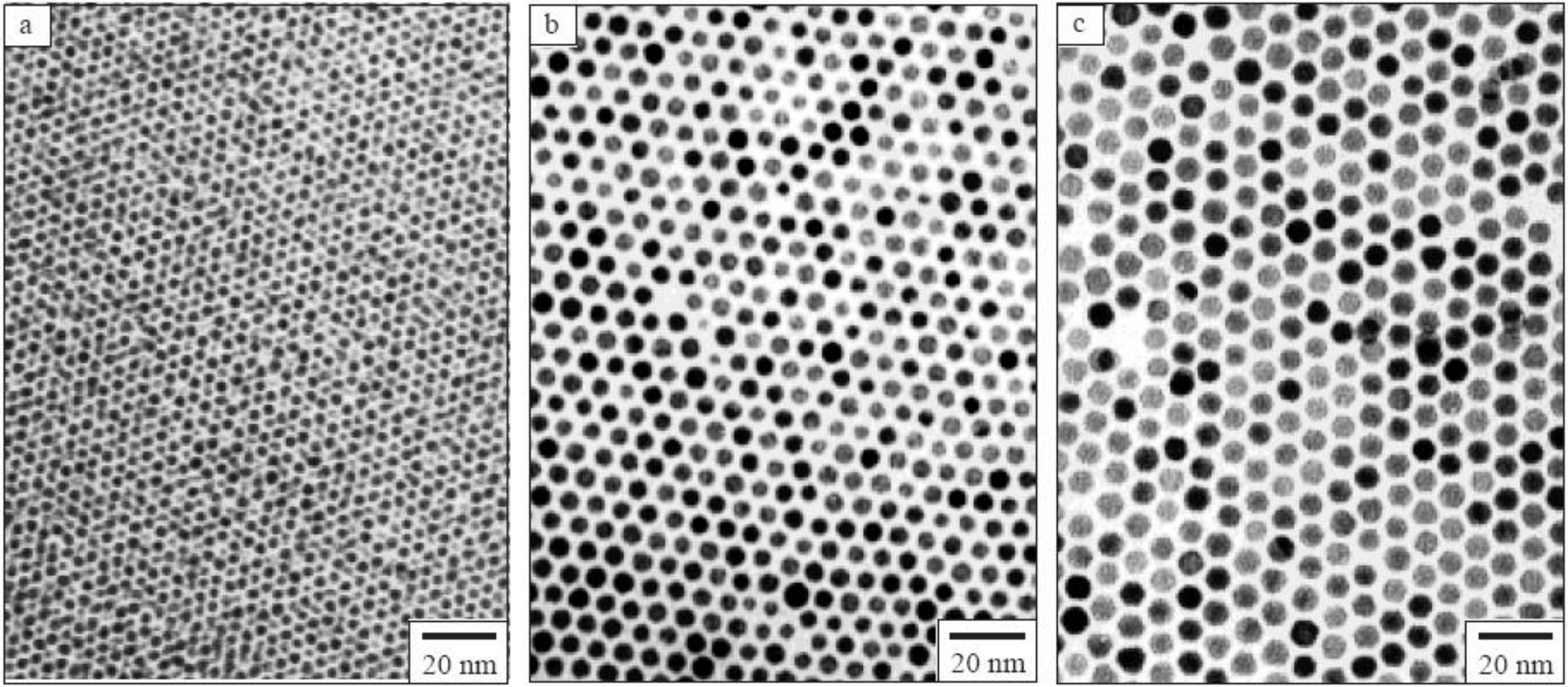
Teranishi, and Miyake, *J. Phys. Chem. B* **2003,** *107,* 2719

See also *Adv. Mater.* **2001**, 13, 1699.

- The initial dodecanethiol-stabilized 1.5 nm sized gold nanoparticles, which were prepared by Brust's two-phase method,
- Increased in size to 3.4, 5.4, and 6.8 nm

  by heating at 150, 190, and 230 °C, respectively.
- Thermolysis of crude preparations of Brust's AuNPs without removing the phase-transfer reagent, tetraoctylammonium bromide, to 150-25 °C led to an increase of the particle sizes to 3.4-9.7 nm.


(a)
20nm
(b)
20nm
(c)
20nm
1.5 nm (σ = 0.2 nm)
3.4 nm (σ = 0.3 nm)
5.4 nm (σ = 0.7 nm)
Distribution / %
(d)
20nm
(e)
20nm
6.8 nm (σ = 0.5 nm)
9.7 nm (σ = 0.9 nm)
Diameter / nm

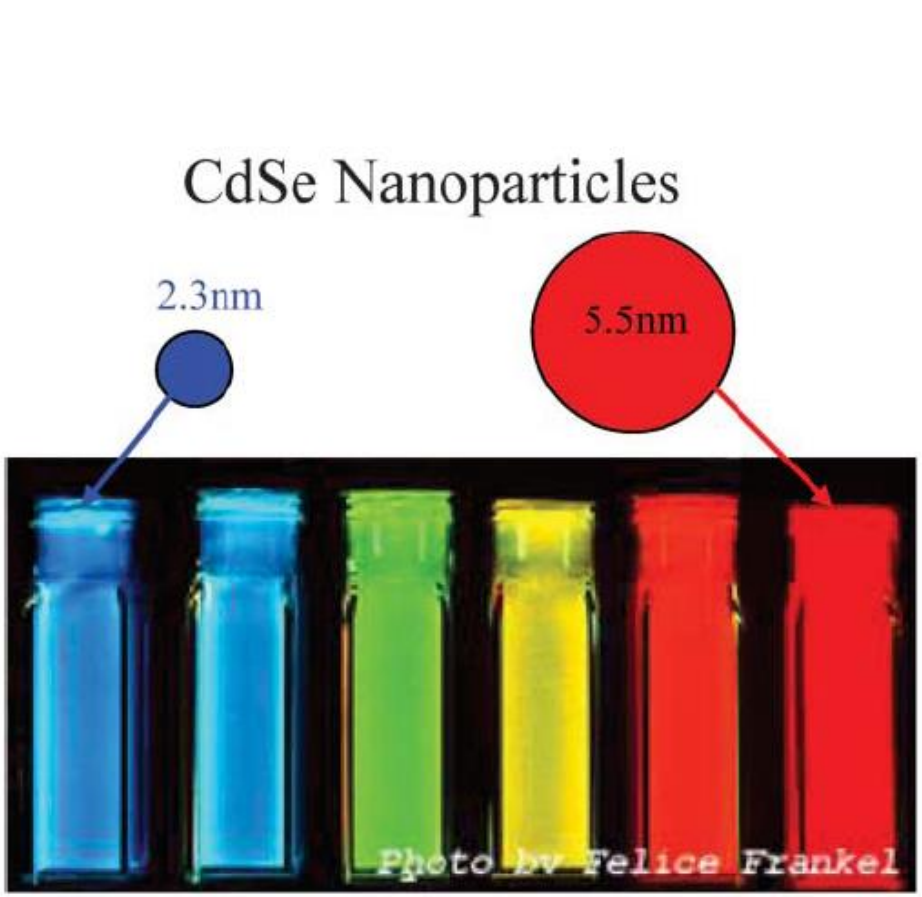


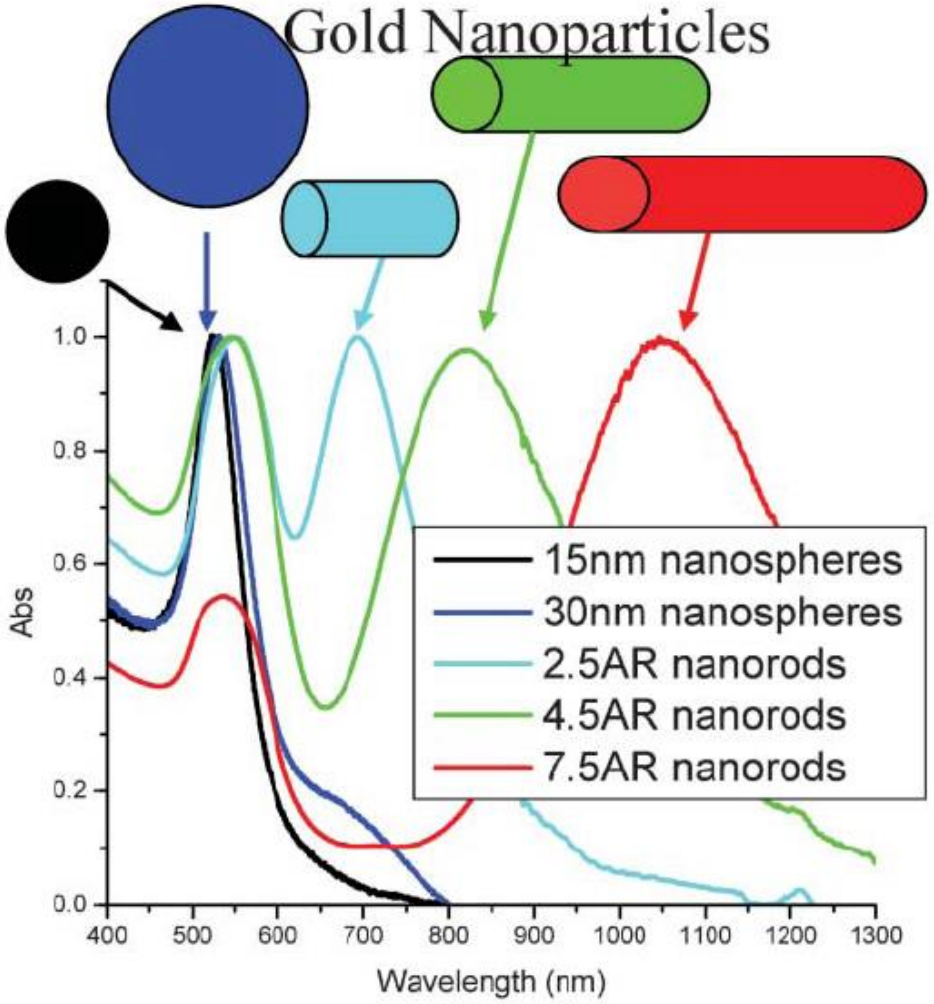
Adv. Mater. **2001**, 13, 1699.

Temp. [°C]	MD [nm]	SD [nm]	[Au] [wt.-%]
–	1.5	0.2	71.9
150	3.4	0.3	79.7
190	5.4	0.7	93.3
230	6.8	0.5	94.7

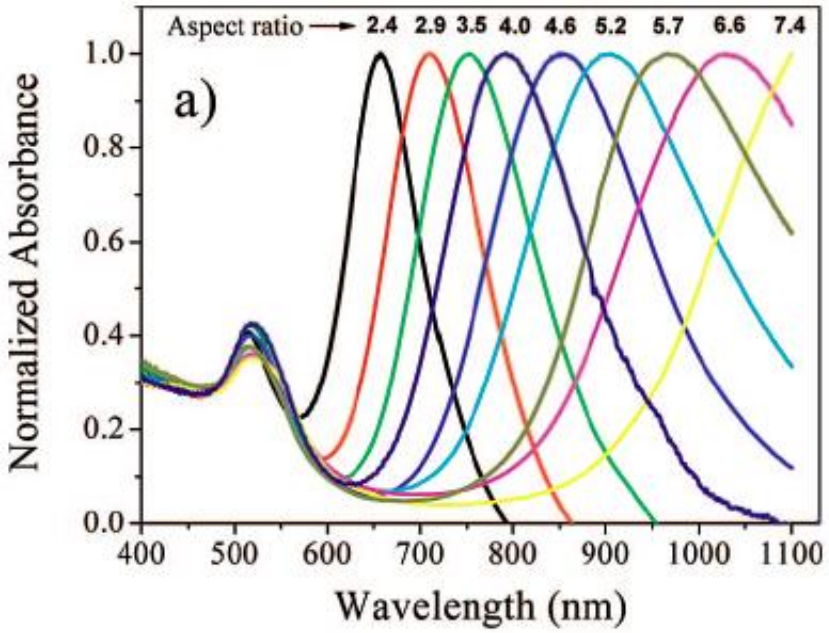
QD's vs. Metal Nanoparticles

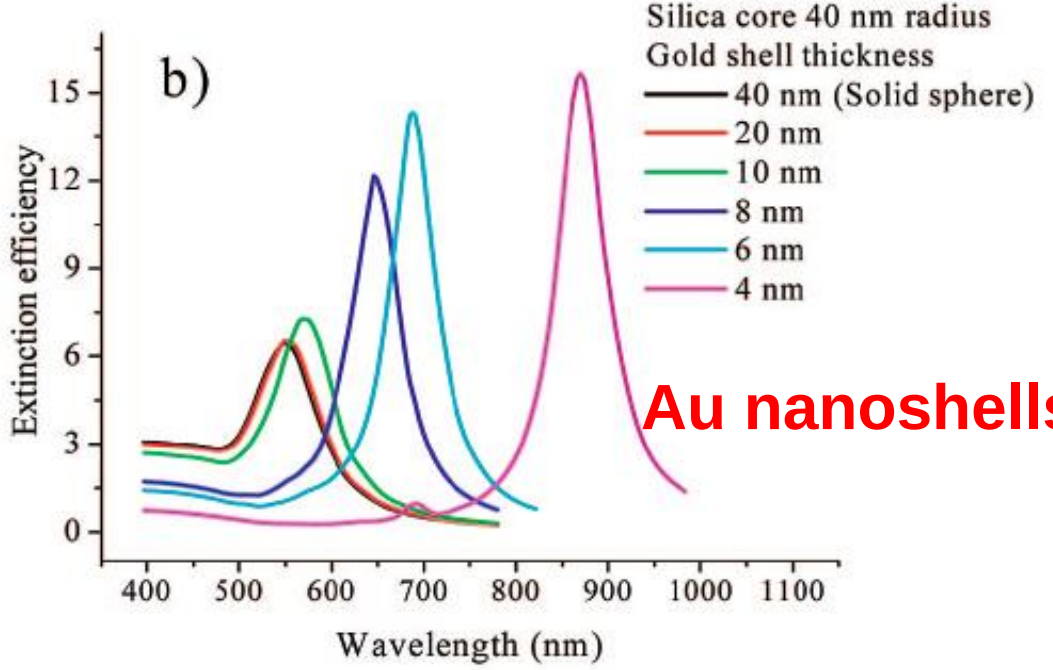
- This absorption does not derive from transitions between quantized energy states.
- Instead, in metal particles, collective modes of motion of the electron gas can be excited. They are referred to as surface plasmons.
- Freely mobile electrons are trapped in such metal boxes and show a characteristic collective oscillation frequency of the plasma resonance, giving rise to the so-called surface plasmon resonance (SPR) band observed near 530 nm in the 5-20-nm-diameter range.
- The size dependence of the plasmon frequency is negligible: No shift in Absorption maximum for colloidal gold nanocrystals in the range between 5 and 30 nm.





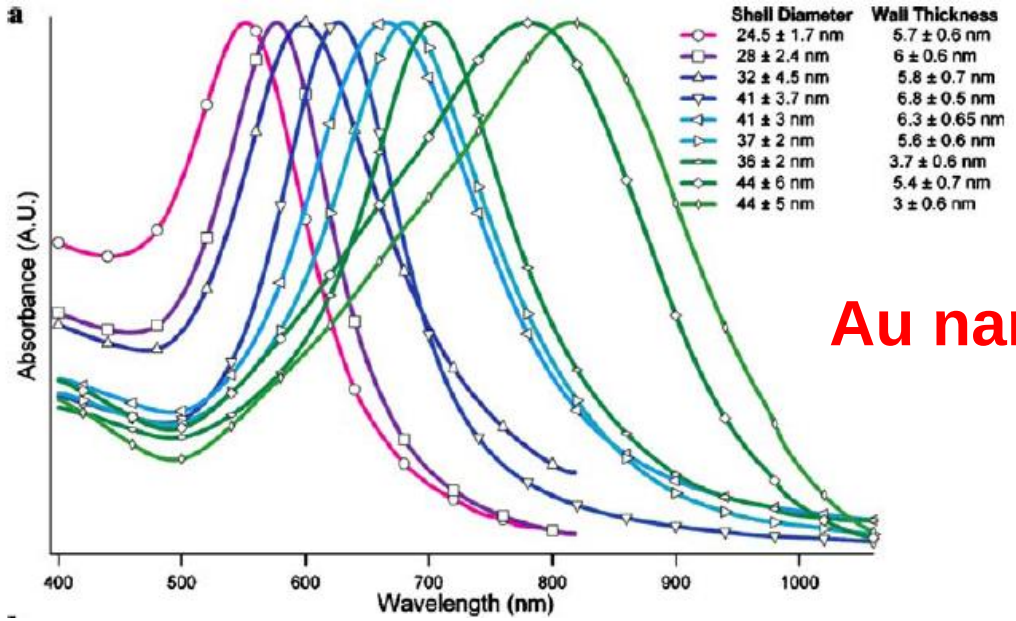
S. Eustis, M. A. El-Sayed, *Chem. Soc. Rev.* **2006**, *35*, 209-217.





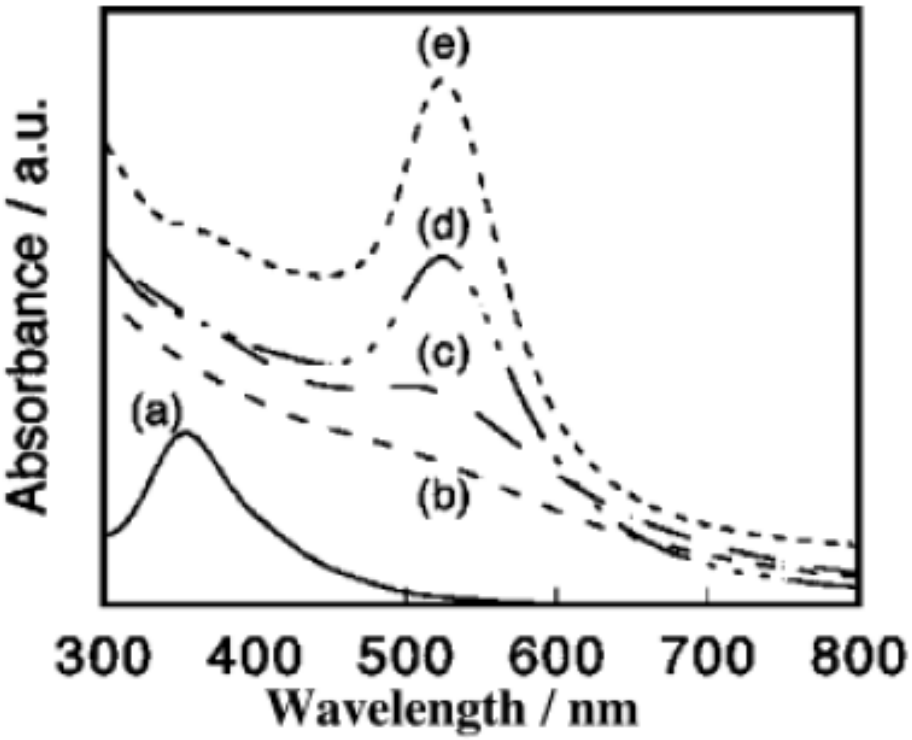
Au nanoshells

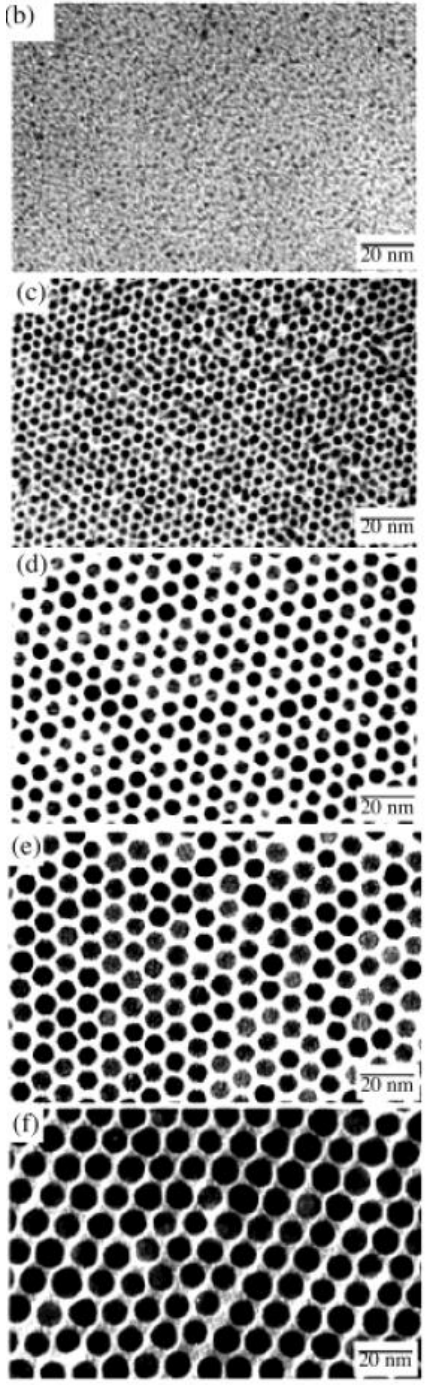
Au nanorods

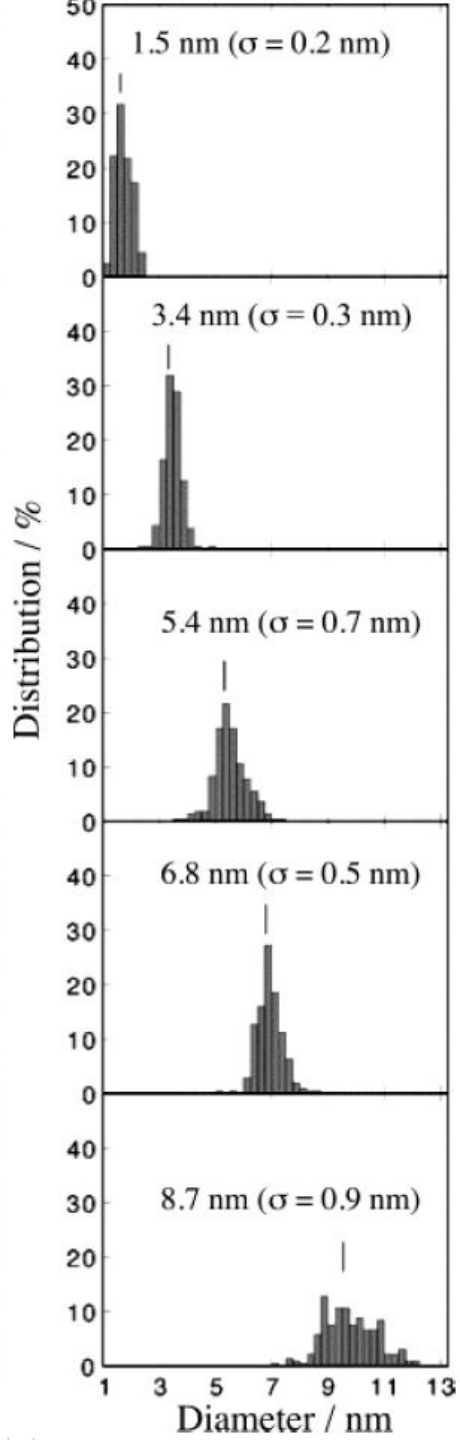


Au nanoshells

Mostafa A. El-Sayed, *Acc. Chem. Res.*, **2008**, 41 (12), 1578-1586.


Absorbance / a.u.
(a)
(b)
(c)
(d)
(e)
300
400
500
600
700
800
Wavelength / nm


(b)
20 nm
(c)
20 nm
(d)
20 nm
(e)
20 nm
(f)
20 nm

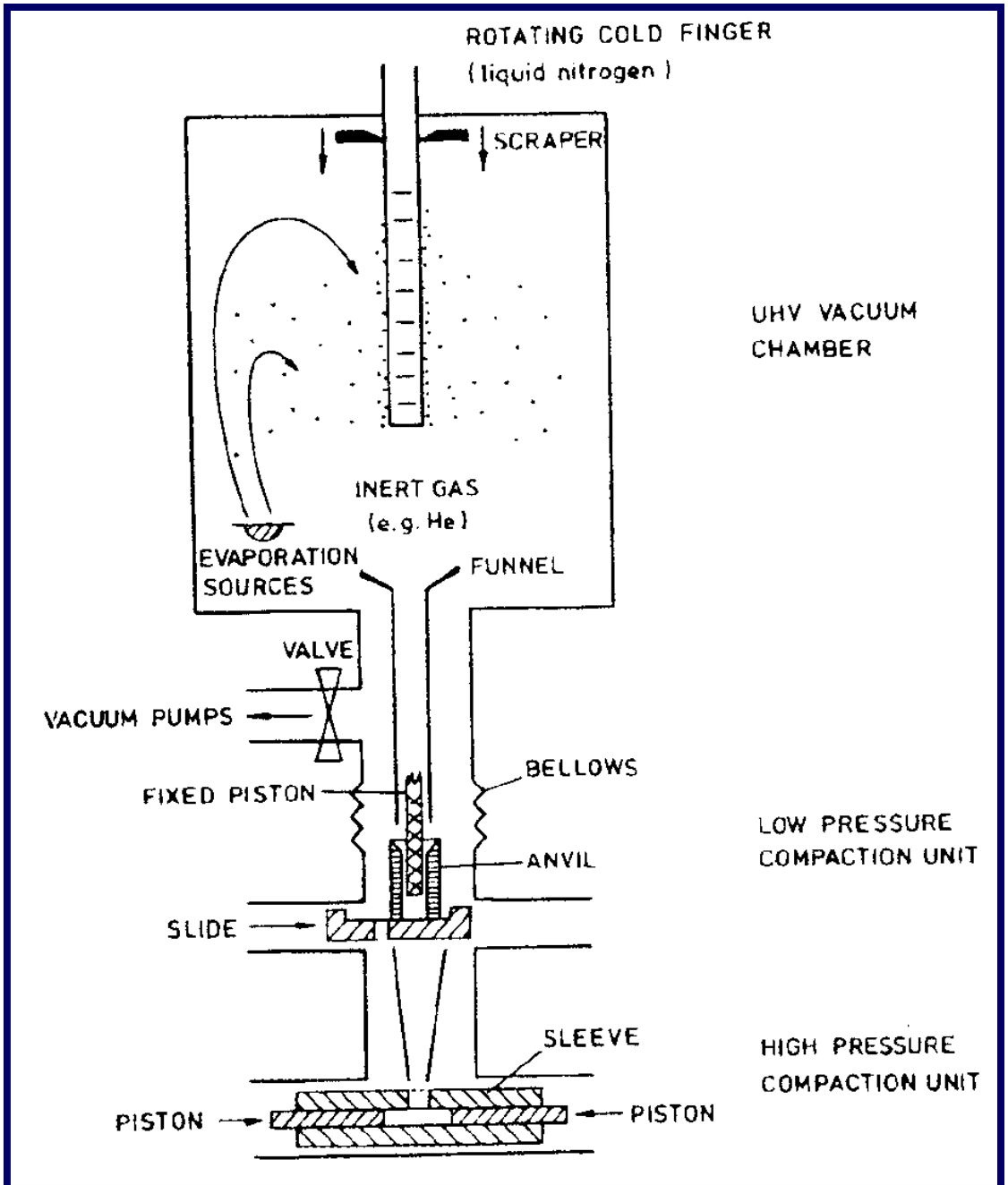

1.5 nm (σ = 0.2 nm)
3.4 nm (σ = 0.3 nm)
5.4 nm (σ = 0.7 nm)
6.8 nm (σ = 0.5 nm)
8.7 nm (σ = 0.9 nm)
Distribution / %
0
10
20
30
40
50
1
3
5
7
9
11
13
Diameter / nm

Gram-Scale Synthesis of Monodisperse Gold Colloids by the Solvated Metal Atom Dispersion Method and Digestive Ripening and Their Organization into Two- and Three-Dimensional Structures

Kenneth J. Klabunde (Kansas State University)

JACS 2002, 124, 2305

Vapor Condensation Method



Gleiter, H.
Adv. Mater. **1992**, 4, 474

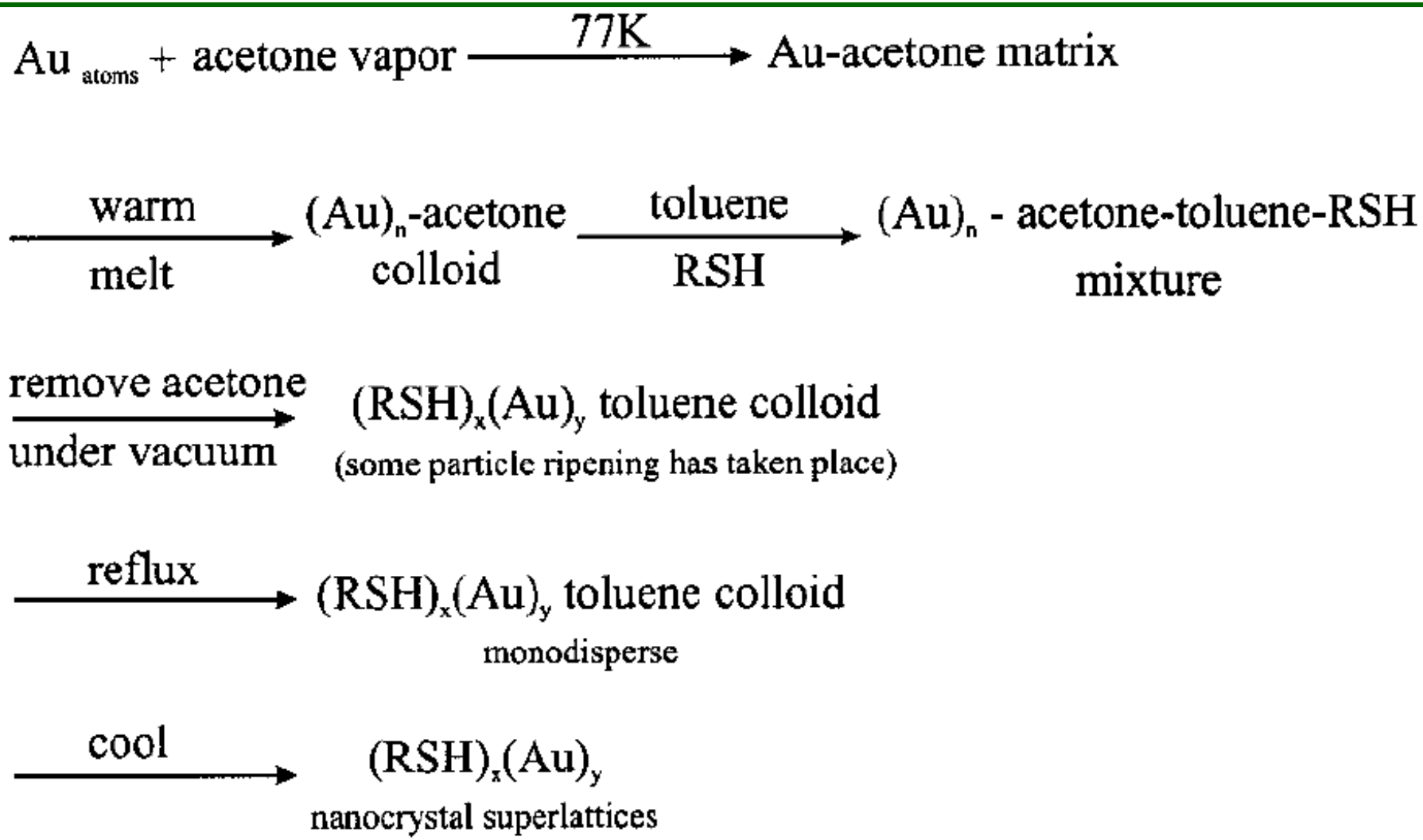
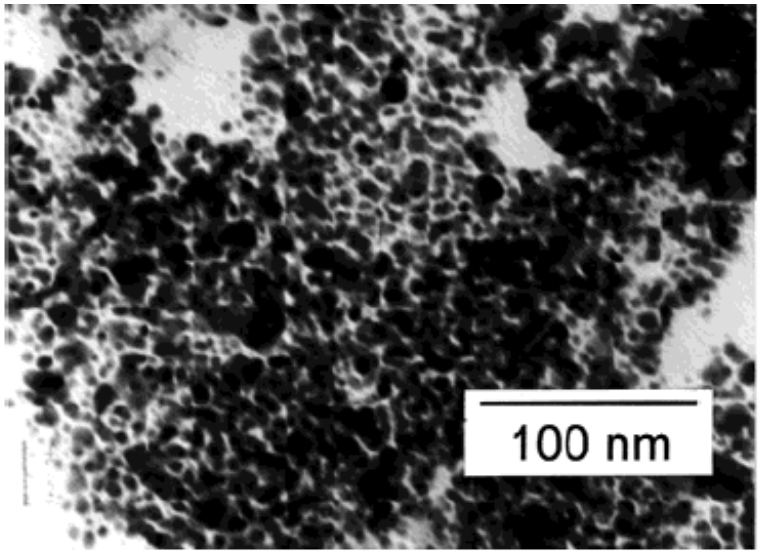
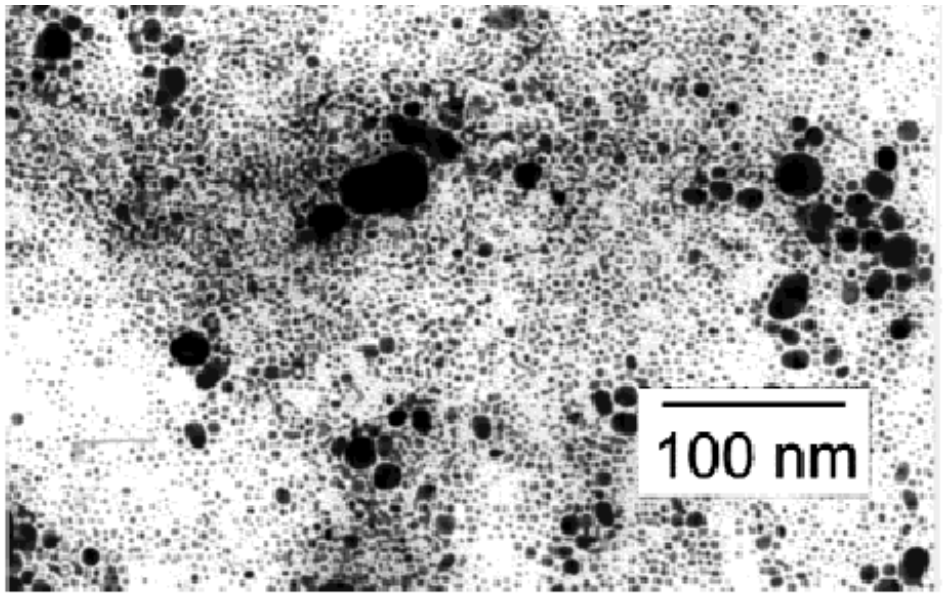


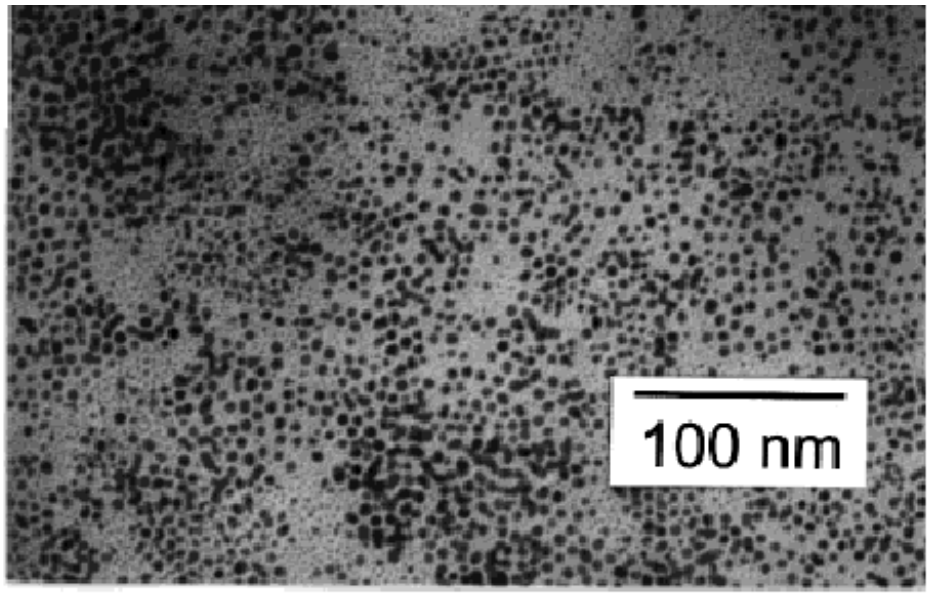
Figure 1. Flow diagram of synthetic steps for preparation of nanocrystal superlattices.



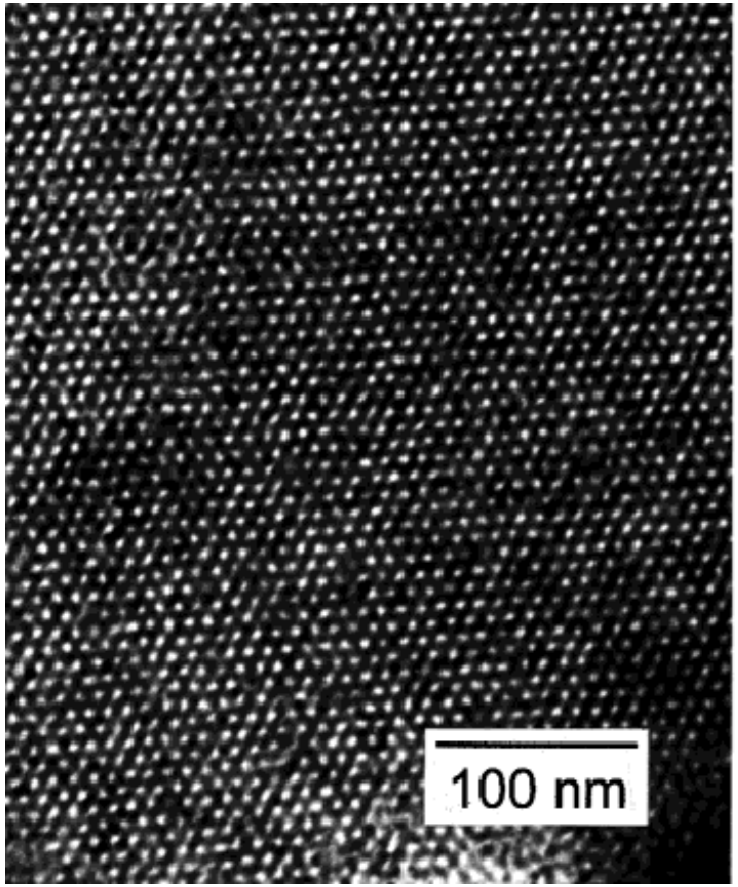
Au-acetone-toluene-thiol colloid
Initial NP: 5 ~ 40 nm
polydisperse

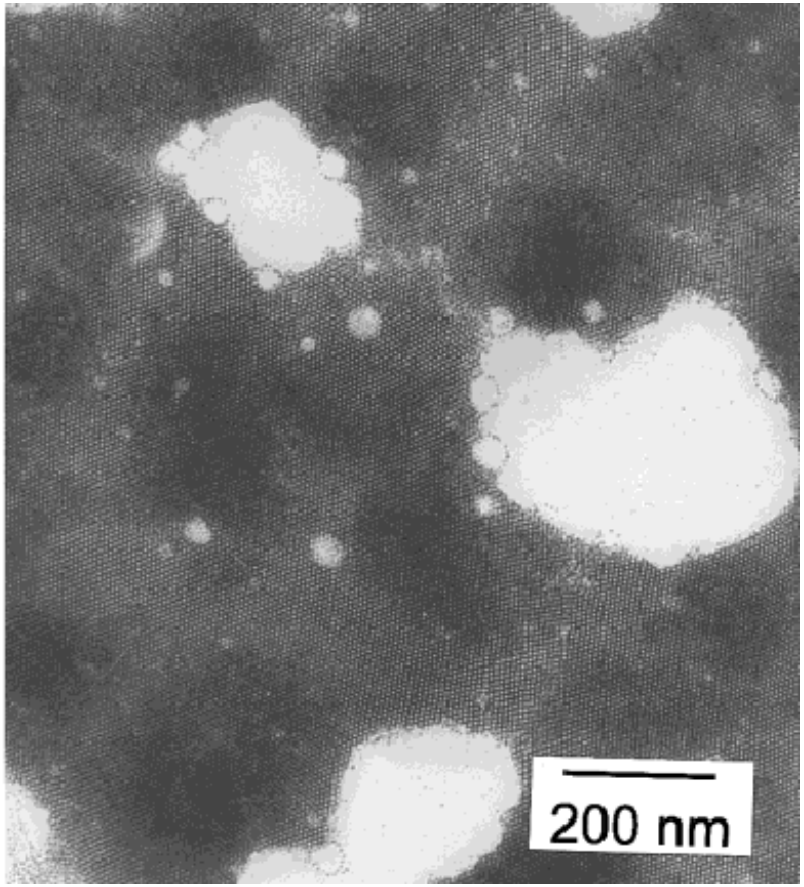
Figure 2. TEM micrograph of gold particles from Au−acetone−toluene-thiol colloid (colloid 1).





Au-toluene-thiol colloid



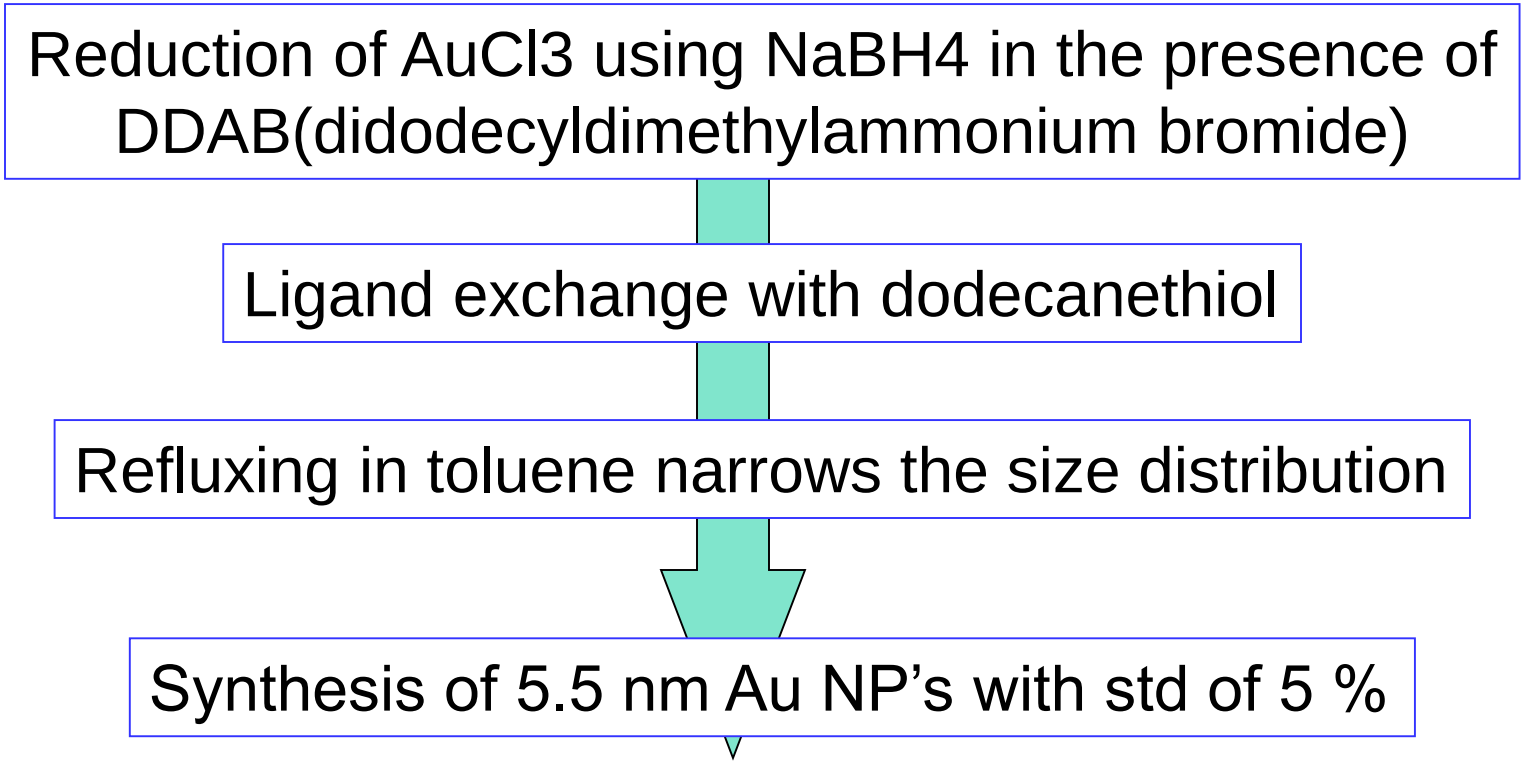


1 day digestive ripening process; 3 month

Long-range ordered Nanocrystal Superlattices on SiN

JPC-B 2001, 105, 3353 by Klabunde at Kansas SU.

Synthesis of Monodisperse Au Nanoparticles



•Evaporation of solvent induces superlattice formation

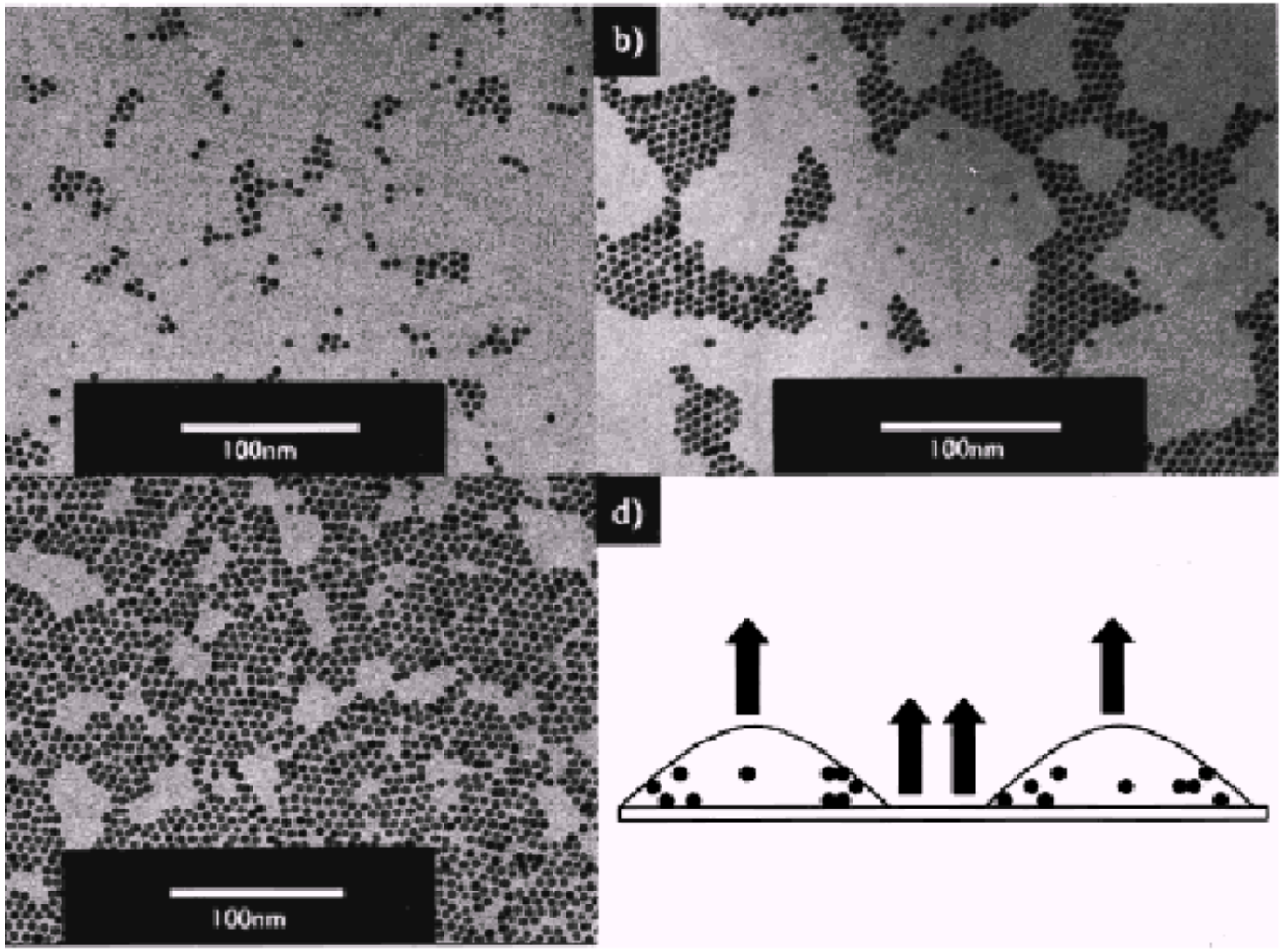
• Few hundreds of nanometers

• Polarity of the solvent affects wetting properties, influencing the aggregation of nanocrystals on the surface (PRL 198, 80, 3531)

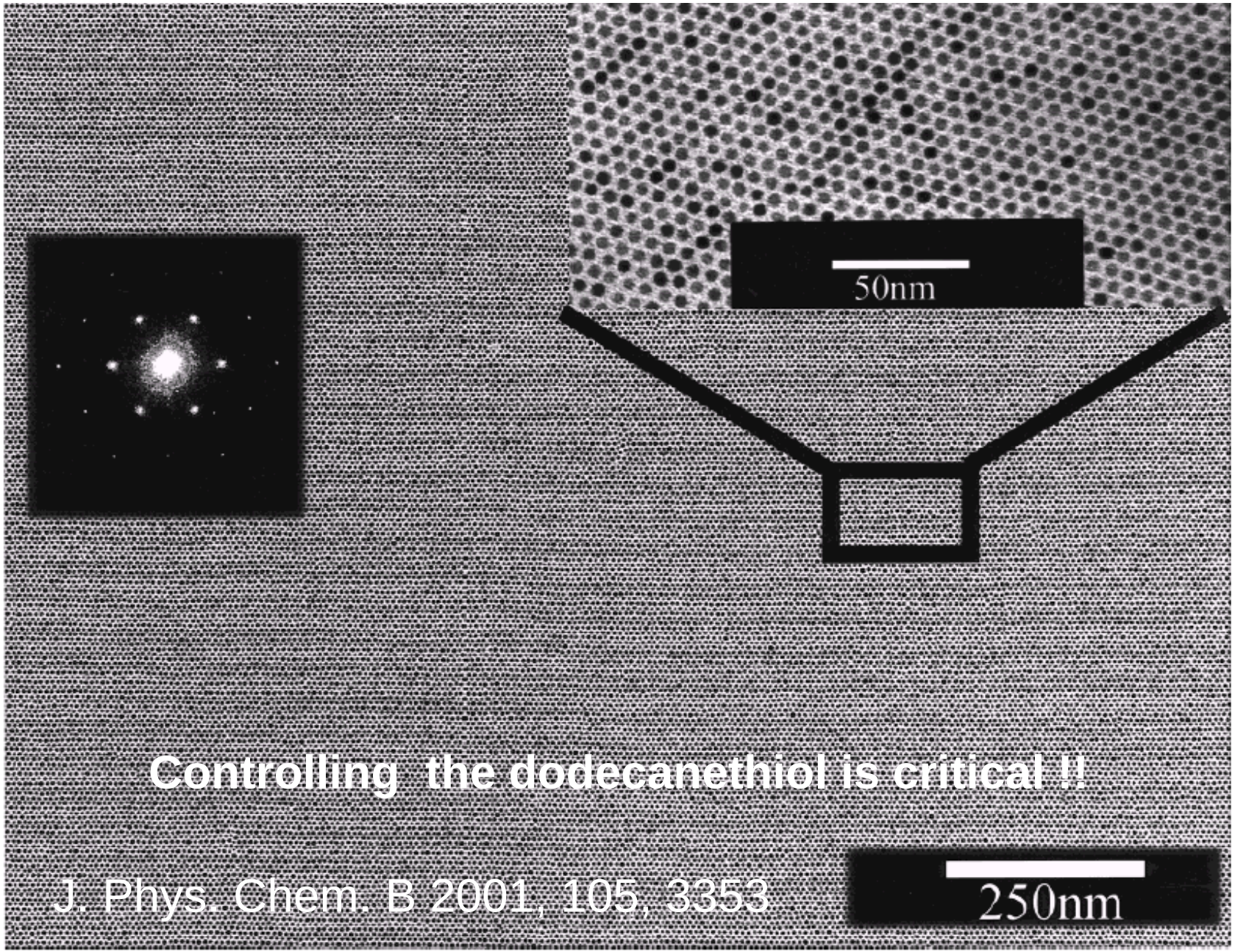
• Competition of 2D superlattice formation and solvent dewetting

• Control of dewetting process to get long-range ordered superlattice With domain size of several microns.

When pure toluene was used, evaporation is too fast to form superlattice



Superlattice formation in micrometer-scale

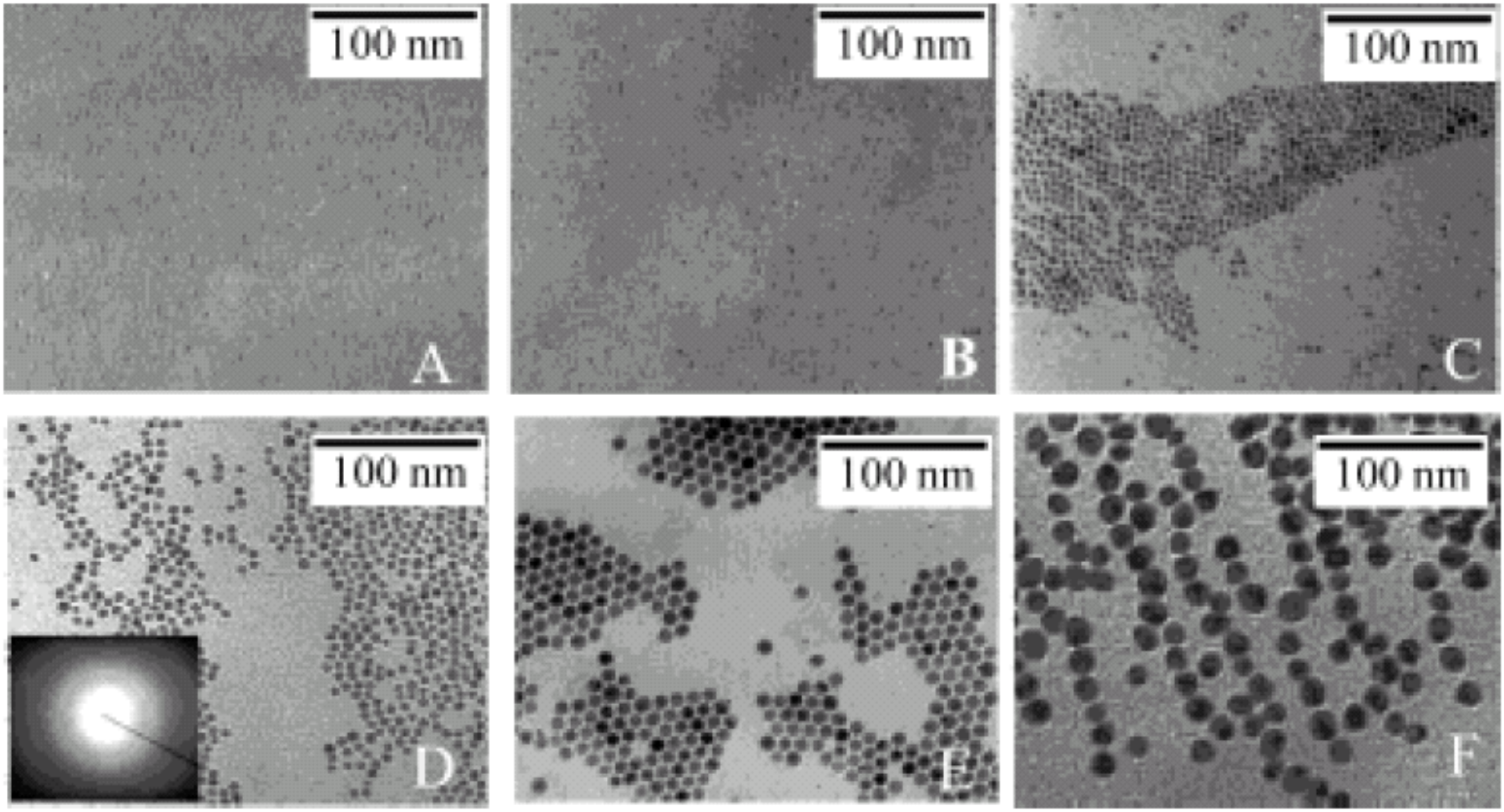


- Control of particle-particle and particle-substrate interaction to self-assemble superlattice structures upon drying
- Rapid dewetting of volatile solvent: bad for superlattice formation
- Long-range superlattice formation through increasing the concentration of nonvolatile dodecanethio ligand.
- Monolayer and bilayer control by NP concentration

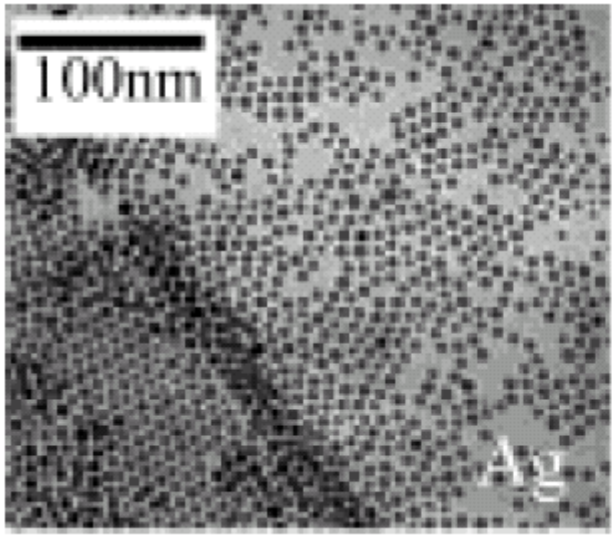
X. G. Peng, *J. AM. CHEM. SOC.* 2003, 125, 14280.

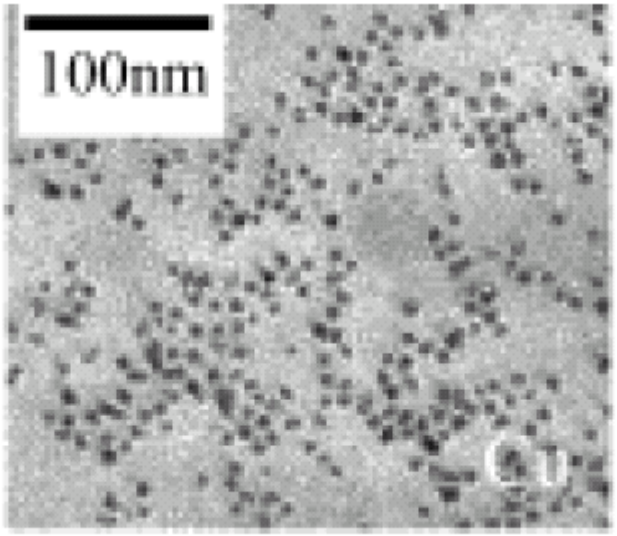
Single-phase system Synthesis

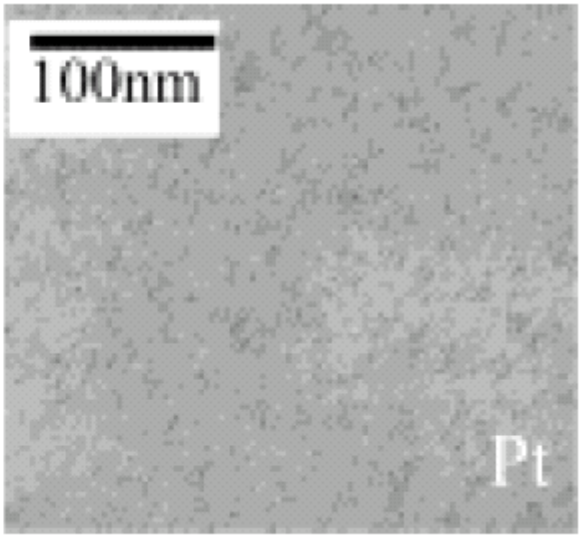
- AuCl3, Ag(CH3COO), Cu(CH3COO)2, or PtCl4 was dissolved in toluene with an ammonium surfactants.
- Either tetrabutylammonium borohydride (TBAB) or its mixture with hydrazine in toluene was used as reducing reagents.
- Fatty acids or aliphatic amines were added as ligands.


100 nm
A
100 nm
B
100 nm
C
100 nm
D
100 nm
E
100 nm
F

Ag, Cu, and Pt nanocrystals







Applications of

Gold Nanoparticles

See references 506 – 517 of
Chem. Rev. **2004,** *104,* 293-346.

Surface Plasmon Resonance (SPR)

- Freely mobile electrons are trapped in such metal boxes and show a characteristic collective oscillation frequency of the plasma resonance, giving rise to the so-called surface plasmon resonance (SPR) band observed near 530 nm in the 5-20-nm-diameter range.
- The deep-red color of AuNP sols in water and glasses reflects the surface plasmon resonance (SPR), a broad absorption band in the visible region around 520 nm.
- The SPR is due to the **collective oscillations of the electron gas at the surface of nanoparticles** (6s electrons of the conduction band for AuNPs) that is correlated with the electromagnetic field of the incoming light, i.e., the excitation of the coherent oscillation of the conduction band.

Main characteristics of SPB

(i) its position around 520 nm;

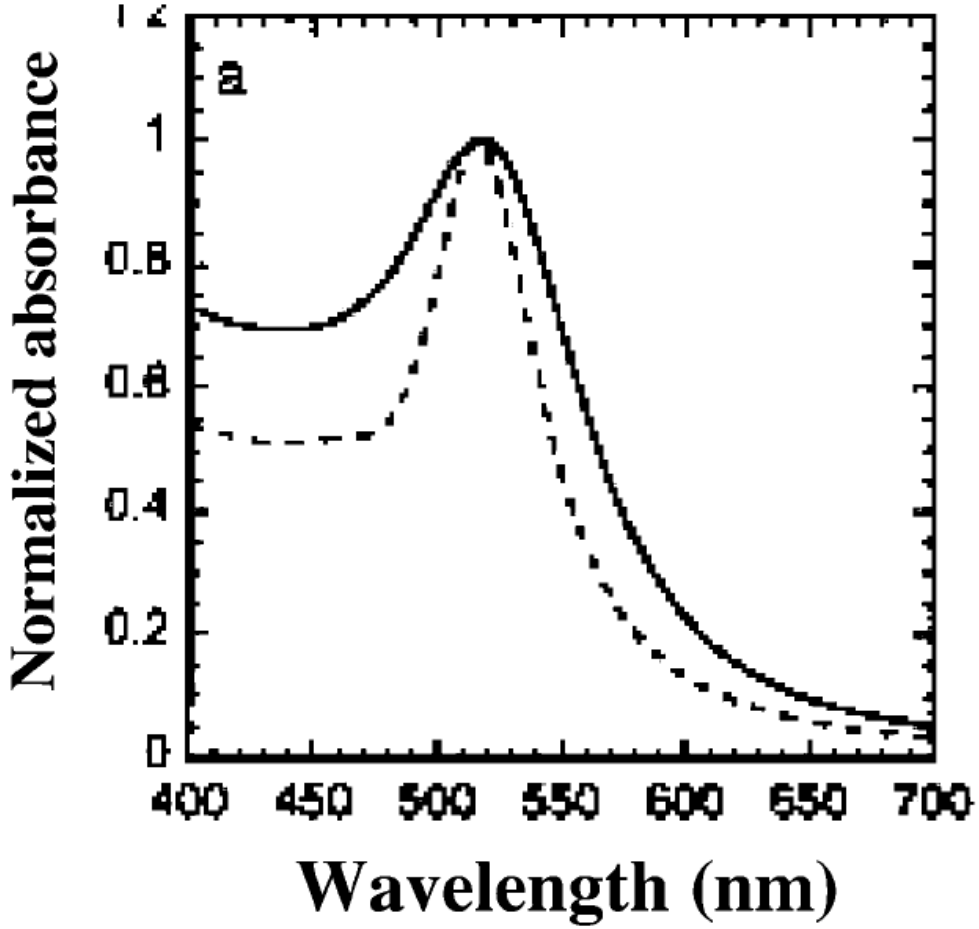
(ii) its sharp decrease with decreasing core size for AuNPs with 1.4-3.2-nm core diameters due to the onset of quantum size effects that become important.

(iii) SPR is absent for AuNPs with core diameter less than 2 nm, as well as for bulk gold.

(iv) For AuNPs of mean diameter of 9, 15, 22, 48, and 99 nm, the SPB maximum λmax was observed at 517, 520, 521, 533, and 575 nm, respectively, in aqueous media.

(v) The SPR maximum and bandwidth are also influenced by the particle shape, medium dielectric constant, and temperature.

Optical absorption spectra of 8.3 nm Au nanoparticles in water



Programmed Materials Synthesis with DNA

James J. Storhoff and Chad A. Mirkin*

Chem. Rev. **1999,** *99,* 1849-1862

ORIGINAL PAPER:

DNA Based Method for Rationally Assembling Nanoparticles Into Macroscopic Materials.

Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J.

Nature **1996**, *382*, 607-609.

A DNA-based method for rationally assembling nanoparticles into macroscopic materials

Chad A. Mirkin, Robert L. Letsinger, Robert C. Mucic & James J. Storhoff

Department of Chemistry, Northwestern University, Evanston, Illinois 60208, USA

COLLOIDAL particles of metals and semiconductors have potentially useful optical, optoelectronic and material properties[1–4] that derive from their small (nanoscopic) size. These properties might lead to applications including chemical sensors, spectroscopic enhancers, quantum dot and nanostructure fabrication, and microimaging methods[2–4]. A great deal of control can now be exercised over the chemical composition, size and polydispersity[1,2] of colloidal particles, and many methods have been developed for assembling them into useful aggregates and materials. Here we describe a method for assembling colloidal gold nanoparticles rationally and reversibly into macroscopic aggregates. The method involves attaching to the surfaces of two batches of 13-nm gold particles non-complementary DNA oligonucleotides capped with thiol groups, which bind to gold. When we add to the solution an oligonucleotide duplex with 'sticky ends' that are complementary to the two grafted sequences, the nanoparticles self-assemble into aggregates. This assembly process can be reversed by thermal denaturation. This strategy should now make it possible to tailor the optical, electronic and structural properties of the colloidal aggregates by using the specificity of DNA interactions to direct the interactions between particles of different size and composition.

Previous assembly methods have focused on the use of covalent 'linker' molecules that possess functionalities at opposing ends with chemical affinities for the colloids of interest. One of the most successful approaches to date[5] has involved the use of gold colloids and well established thiol adsorption chemistry[6,7]. In this approach, linear alkanedithiols were used as the particle linker molecules. The thiol groups at each end of the linker molecule covalently attach themselves to the colloidal particles to form aggregate structures. The drawbacks of this method are that the process is difficult to control and the assemblies are formed irreversibly. Methods for systematically controlling the assembly process are needed if the materials properties of these unusual structures are to be exploited fully.

Our oligonucleotide-based method allows the controlled and reversible assembly of gold nanoparticles into supramolecular structures. Oligonucleotides offer several advantages over non-biological-based linker molecules. For example, discrete sequences of controlled length and with the appropriate surface binding functionality may be prepared in an automated fashion with a DNA synthesizer. In this way, the molecular recognition properties of the oligonucleotides may be used to trigger the colloidal self-assembly process. The interparticle distances and stabilities of the supramolecular structures generated by this method can be controlled through the choice of oligonucleotide sequence and length, solvent, temperature and supporting electrolyte concentration.

Others also have recognized the utility of DNA for the preparation of new biomaterials and nanofabrication methods. Previous researchers have focused on using the sequence-specific molecular-recognition properties of oligonucleotides to design impressive structures with well defined geometric shapes and sizes[8–18]. The chemistry proposed here focuses on merging the chemistry of DNA with the chemistry of inorganic colloidal materials. In addition to generating materials with properties that are hybrids of their DNA and colloidal precursors, the union of metal-colloid and DNA chemistry offers significant opportunities relative to the construction of pure DNA materials. As noted by Seeman[19], 'the theory of producing DNA [structures] is well ahead of experimental confirmation. It is much easier to design a [structure] than it is to prove its synthesis.' An advantage of the DNA/colloid hybrid materials reported herein is that the assemblies can be characterized easily by transmission electron microscopy (TEM) and/or atomic force microscopy (AFM) as well as spectroscopic methods conventionally used with DNA.

Our approach to using oligonucleotides for the controlled assembly of gold nanoparticles into aggregate macroscopic structures is outlined in Fig. 1. First, 13-nm-diameter Au particles are prepared[2,20]. These particles form a dark red suspension in water, and like thin-film Au substrates[21], they are easily modified with oligonucleotides, which are functionalized with alkane thiols at their 3′ termini. In a typical experiment, one solution of 17 nM (150 µl) Au colloids is treated for 24 h with 3.75 µM (46 µl) 3′-thiol-TTTGCTGA, and a second solution of colloids is treated with 3.75 µM (46 µl) 3′-thiol-TACCGTTG. Note that these oligonucleotides are non-complementary. After treatment with the thiol-capped oligonucleotides, the two colloidal Au solutions are combined, and because of the non-complementary nature of the oligonucleotides, no reaction takes place. A beneficial consequence of capping the colloids with these oligonucleotides is

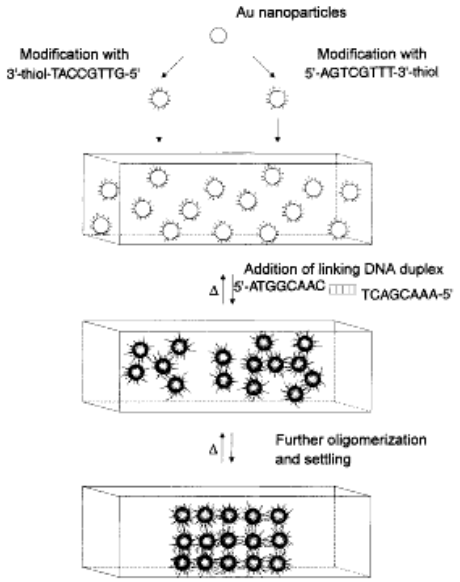


FIG. 1 Scheme showing the DNA-based colloidal nanoparticle assembly strategy (the hybridized 12-base-pair portion of the linking duplex is abbreviated as). If a duplex with a 12-base-pair overlap but with 'sticky ends' with four base mismatches (5′-AAGTCAGTTATACGCGCTAG and 3′-ATATGCGCGATCAAATCACA) is used in the second step, no reversible particle aggregation is observed. The scheme is not meant to imply the formation of a crystalline lattice but rather an aggregate structure that can be reversibly annealed. Δ is the heating above the dissociation temperature of the duplex.

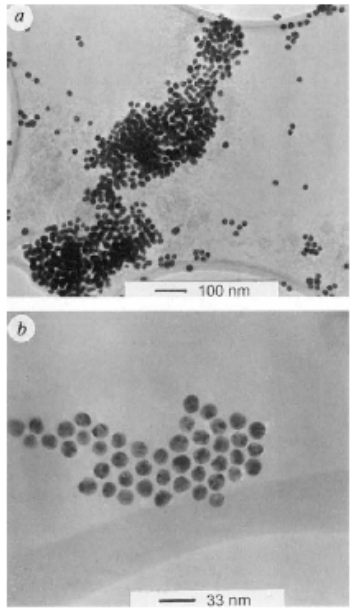


Fig. 4 TEM images of: *a*, an aggregated DNA/colloid hybrid material; *b*, a two-dimensional colloidal aggregate showing the ordering of the DNA-linked Au nanoparticles. Images were taken with a Hitachi 8100 Transmission Electron Microscope.

This work gives entry into a new class of DNA/nanoparticle hybrid materials and assemblies, which might have useful electrical, optical and structural properties that should be controllable through choice of nanoparticle size and chemical composition, and oligonucleotide sequence and length. We note that it should be possible to extend this strategy easily to other noble-metal (for example, Ag, Pt)[22] and semiconductor (for example, CdSe and CdS)[23,24] colloidal nanoparticles with well established surface coordination chemistry. Our initial results bode well for the utility of this strategy for developing new types of biosensing and sequencing schemes for DNA. The Au colloidal particles have large extinction coefficients for the bands that give rise to their colours (Fig. 2). These intense colours, which depend on particle size and concentration and interparticle distance, make these materials particularly attractive for new colorimetric sensing and sequencing strategies for DNA. □

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Organization of 'nanocrystal molecules' using DNA

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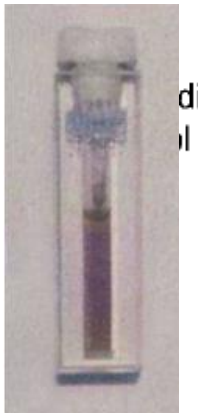
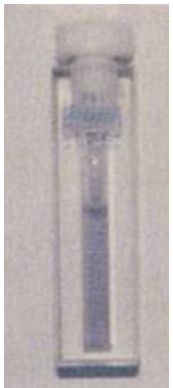
* Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, USA and Molecular Design Institute, Lawrence Berkeley National Laboratory, Berkeley, California 94701, USA
† Howard Hughes Medical Institute, Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, USA

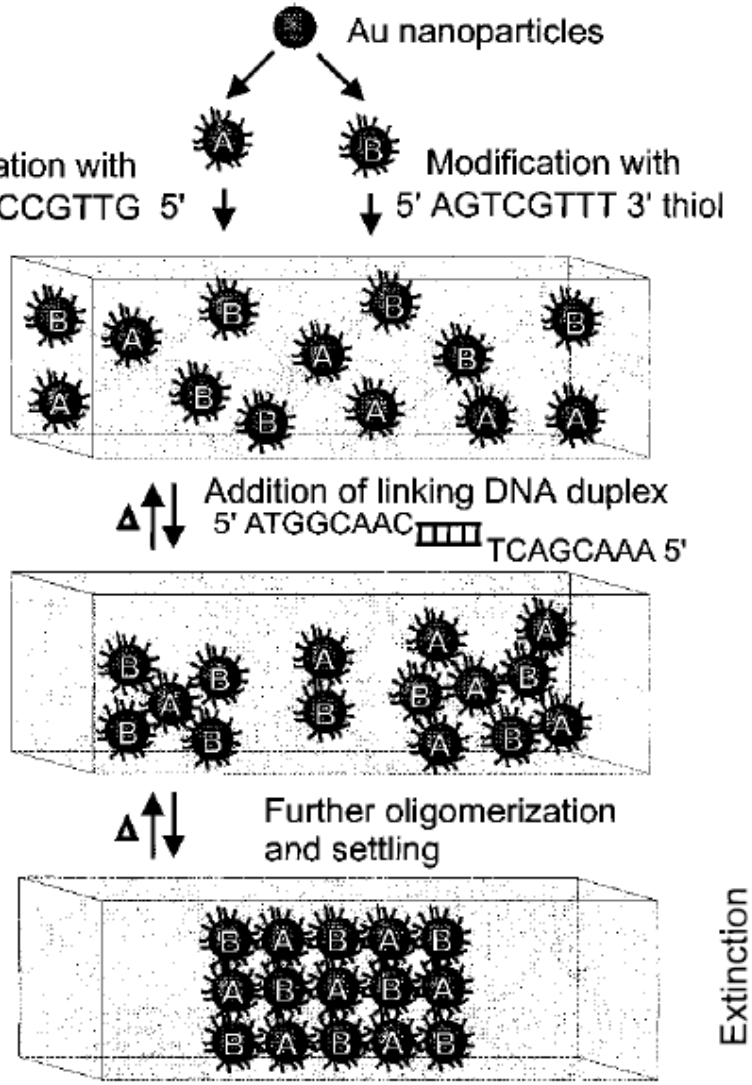
PATTERNING matter on the nanometre scale is an important objective of current materials chemistry and physics. It is driven by both the need to further miniaturize electronic components and the fact that at the nanometre scale, materials properties are strongly size-dependent and thus can be tuned sensitively[1]. In nanoscale crystals, quantum size effects and the large number of surface atoms influence the, chemical, electronic, magnetic and optical behaviour[2–4]. 'Top-down' (for example, lithographic) methods for nanoscale manipulation reach only to the upper end of the nanometre regime[5]; but whereas 'bottom-up' wet chemical techniques allow for the preparation of monodisperse, defect-free crystallites just 1–10 nm in size[6–10], ways to control the structure of nanocrystal assemblies are scarce. Here we describe a strategy for the synthesis of 'nanocrystal molecules', in which discrete numbers of gold nanocrystals are organized into spatially defined structures based on Watson-Crick base-pairing interactions. We attach single-stranded DNA oligonucleotides of defined length and sequence to individual nanocrystals, and these assemble into dimers and trimers on addition of a complementary single-stranded DNA template. We anticipate that this approach should allow the construction of more complex two- and three-dimensional assemblies.

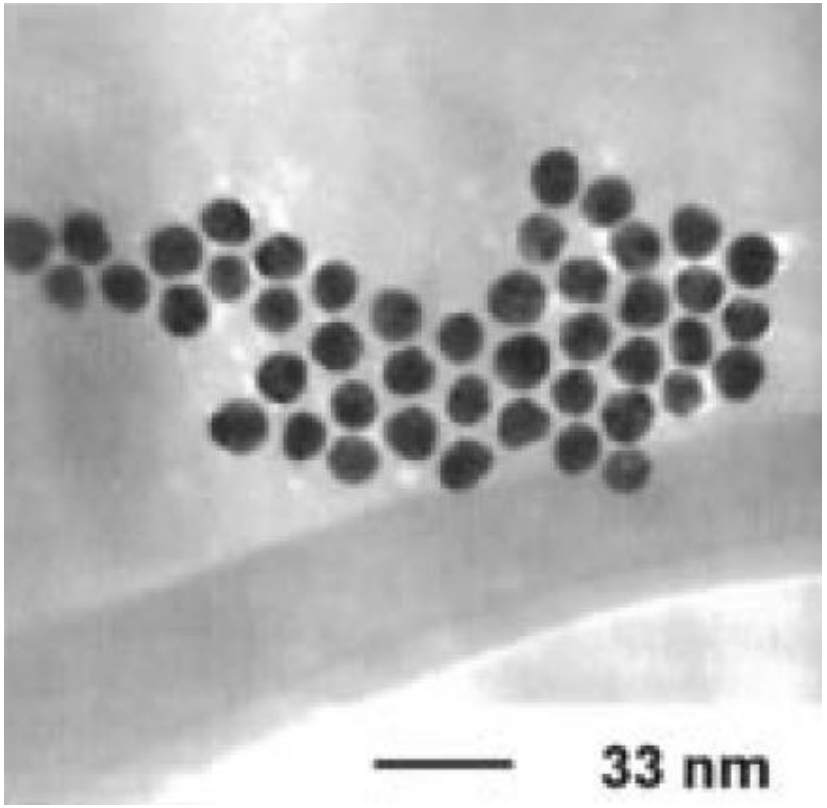
Previous approaches towards the preparation of coupled quantum dots include co-colloids of cadmium selenide–zinc oxide (CdS–ZnO; ref. 11) and cadmium sulphide–silver iodide (CdS–AgI; ref. 12). In addition, small molecule crosslinking agents have been used to synthesize aggregates of Au (ref. 13) and cadmium sulphide linked to titanium oxide (CdS–TiO_2; ref. 14) as well as discrete dimers of cadmium selenide (CdSe; ref. 15). Finally, the collective properties of nanocrystals have been investigated using organic monolayers[16–22] and crystallization[23–28] to generate ordered arrays of inorganic quantum dots. It remains an open question whether self-assembly methods can be employed to generate complex sequences of nanocrystals.

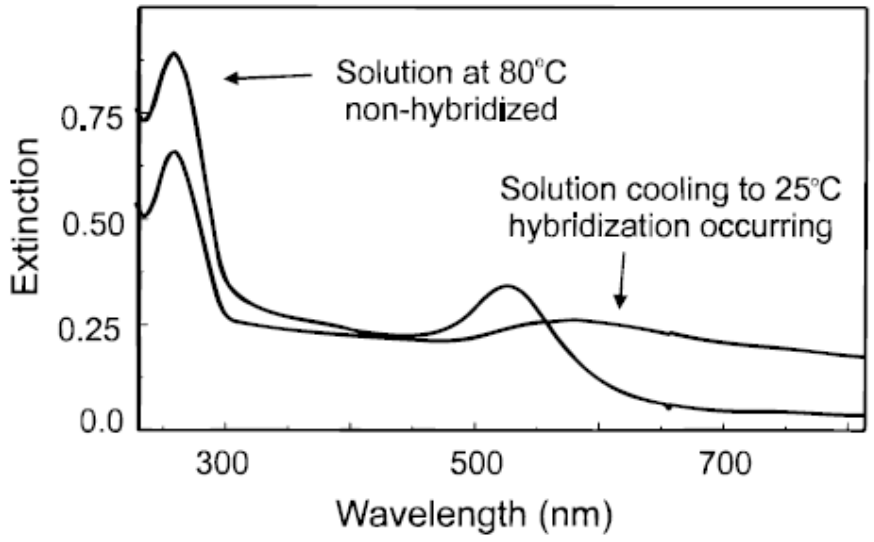
Biological systems are characterized by remarkably complex



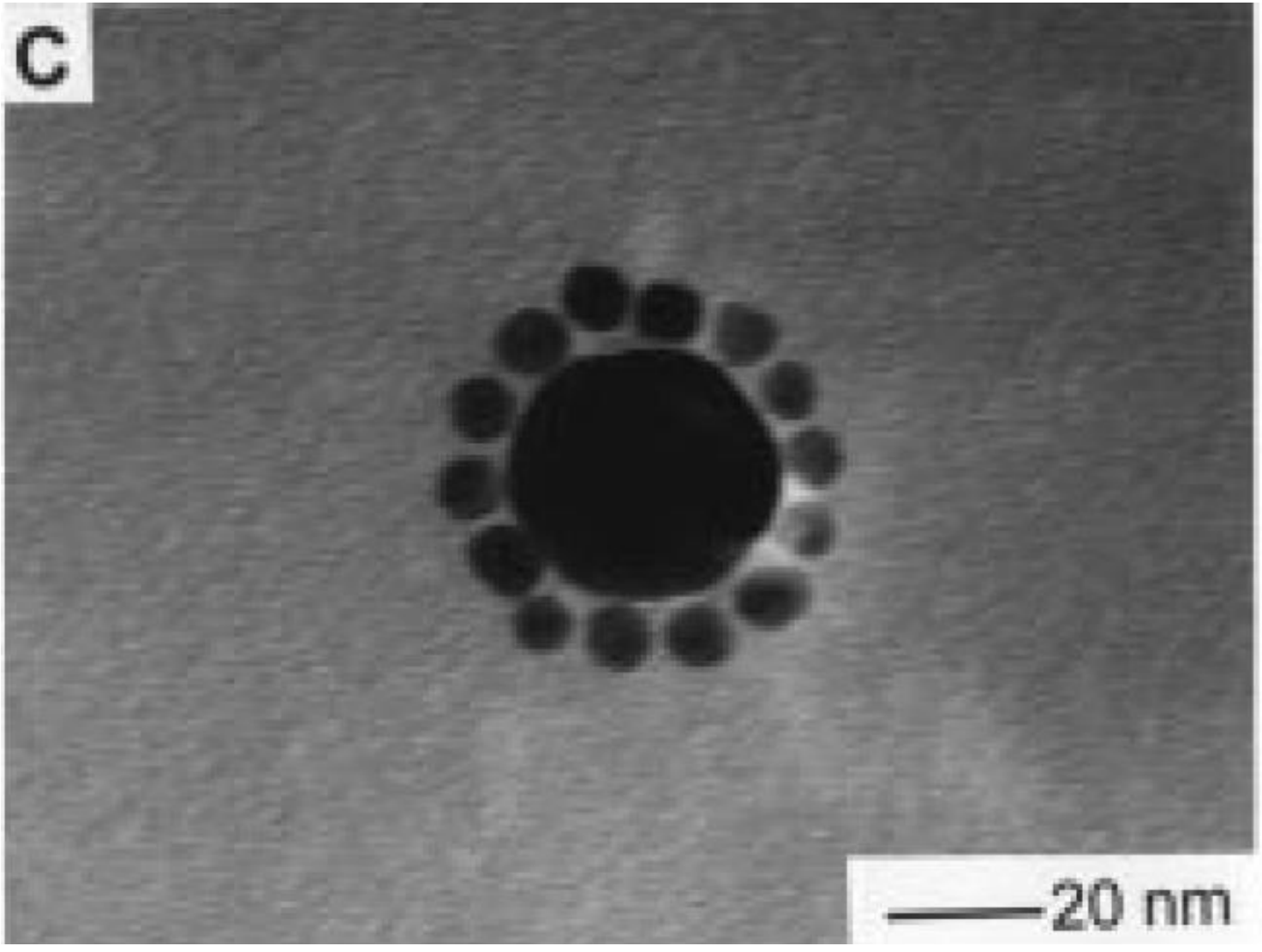


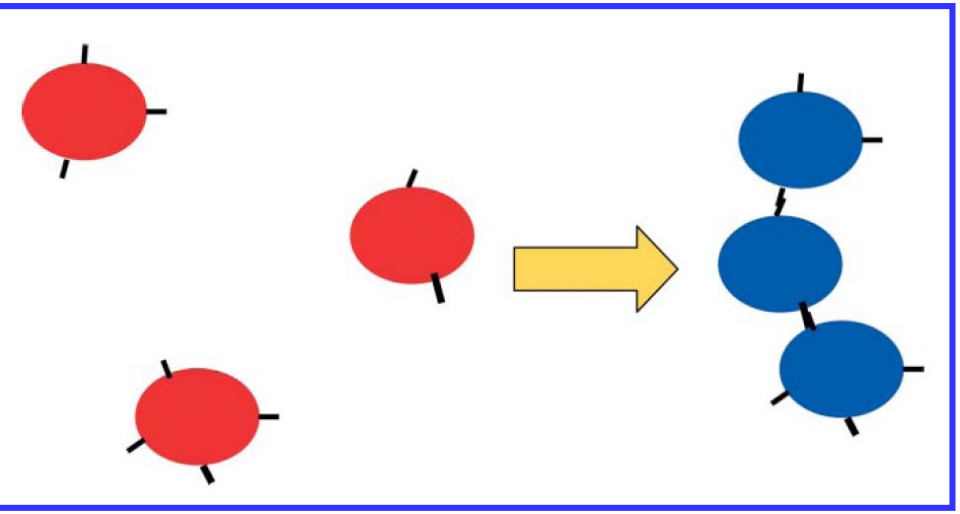

Au nanoparticles
dification with
l TACCGTTG 5'
Modification with
5' AGTCGTTT 3' thiol
Addition of linking DNA duplex
5' ATGGCAAC
TCAGCAAA 5'
Further oligomerization
and settling

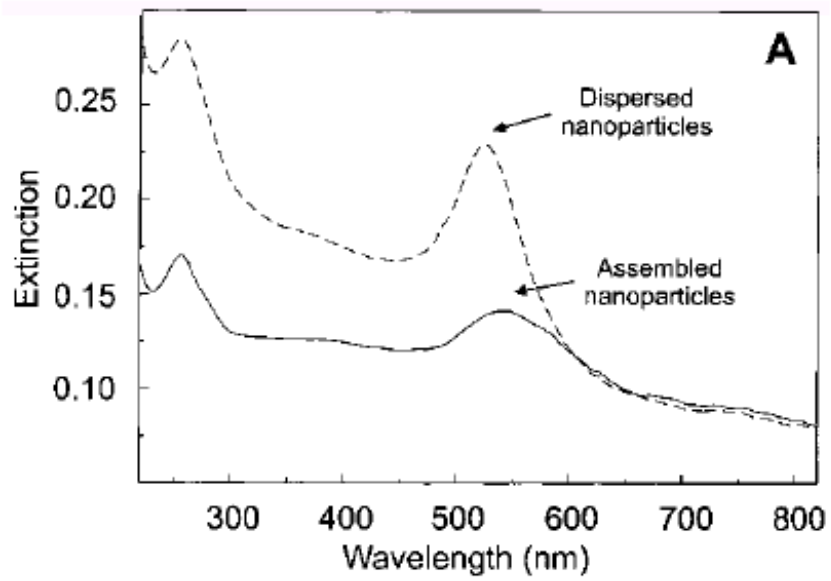

33 nm

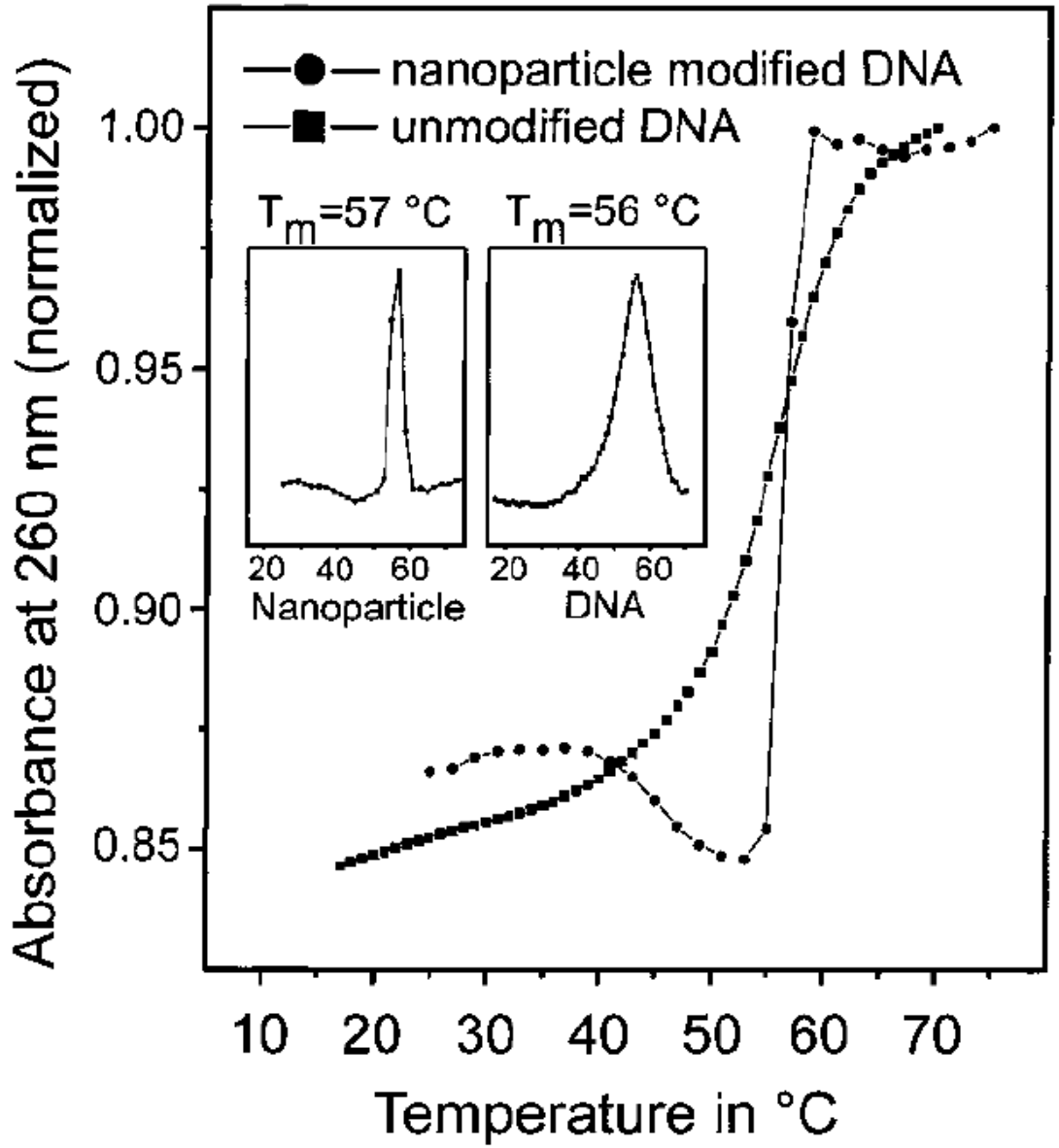

Solution at 80°C
non-hybridized
Solution cooling to 25°C
hybridization occurring
Extinction
0.75
0.50
0.25
0.0
300
500
700
Wavelength (nm)

A nanoparticle “satellite structure” comprised of a 31 nm Au nanoparticle linked through DNA hybridization to several 8 nm Au nanoparticles,






A
Dispersed nanoparticles
Assembled nanoparticles
Extinction
0.25
0.20
0.15
0.10
300
400
500
600
700
800
Wavelength (nm)

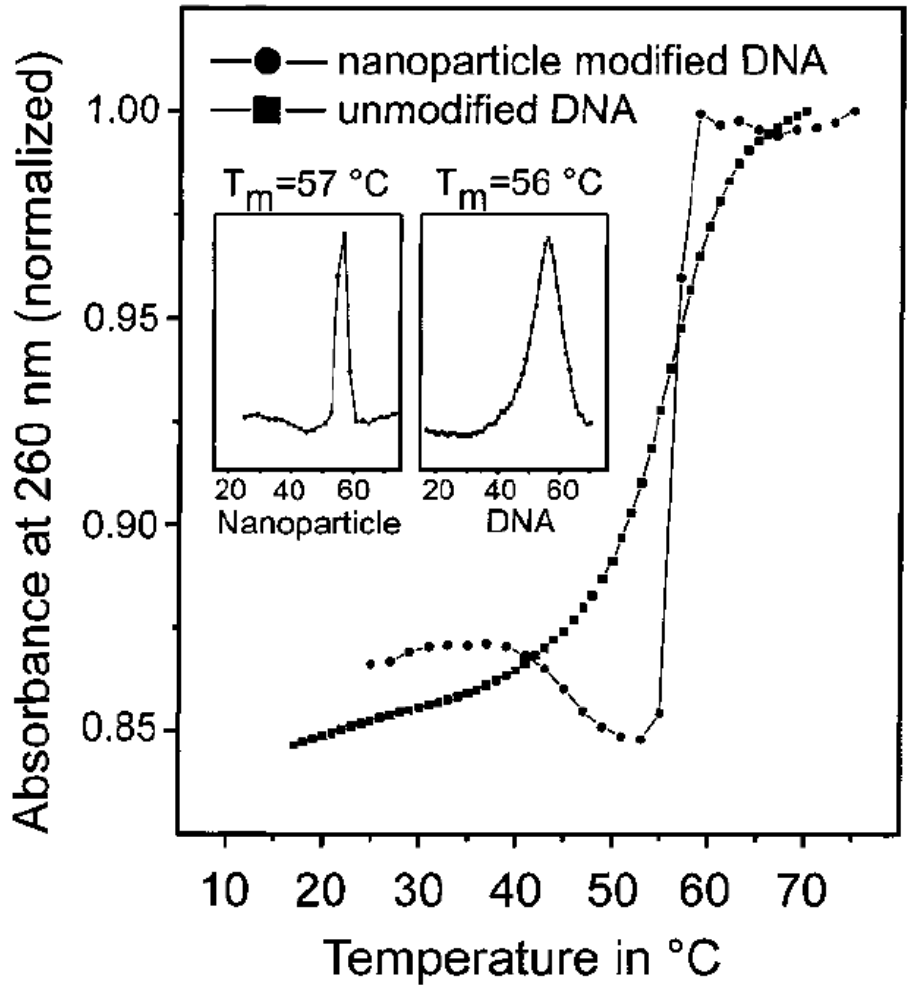

nanoparticle modified DNA
unmodified DNA
T_m=57 °C
T_m=56 °C
20 40 60
Nanoparticle
20 40 60
DNA
Absorbance at 260 nm (normalized)
1.00
0.95
0.90
0.85
10 20 30 40 50 60 70
Temperature in °C

- DNA-AuNPs can bind complementary nucleic acids with a high affinity. In fact, polyvalent particles exhibit binding constants as large as two orders of magnitude greater than the analogous molecular oligonucleotides of the same sequence.➔ from the dense packing and high local concentration of oligonucleotides on the gold surface.

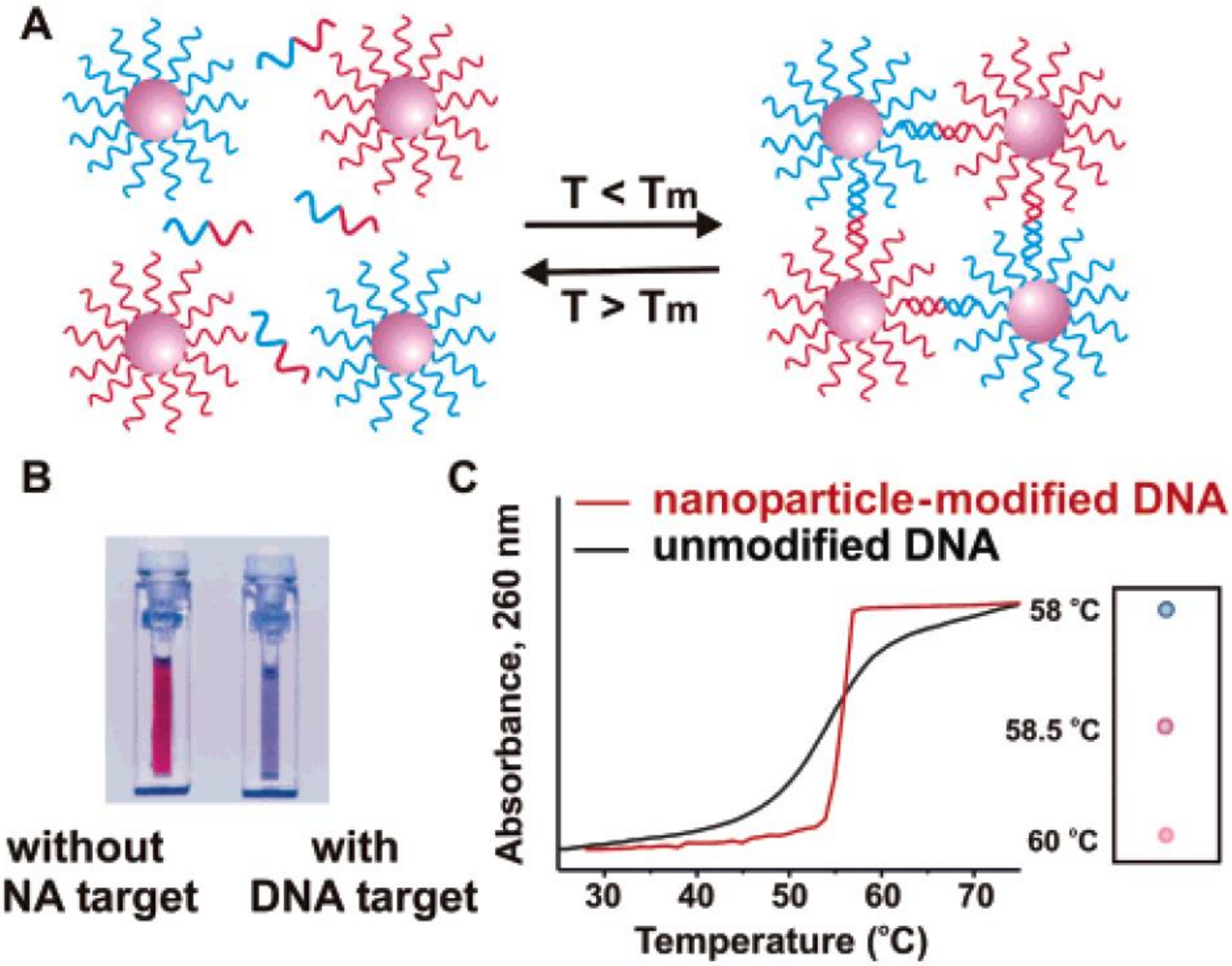
Chad A. Mirkin, *Angew. Chem. Int. Ed.* **2010**, 49, 3280.

Sharp melting behavior of DNA-linked nanoparticles

(1) the formation of an aggregate with many different DNA interconnects
(2) the use of a nanoparticle optical signature rather than a DNA optical signature to map out the melting behavior of the aggregates.



In the presence of complementary target DNA, oligonucleotide-functionalized gold nanoparticles will aggregate.



Nanostructures in Biodiagnostics, N. L. Rosi and Chad A. Mirkin* *Chem. Rev*. **2005**, 105, 1547-1562

Magnetic relaxation switches capable of sensing molecular interactions

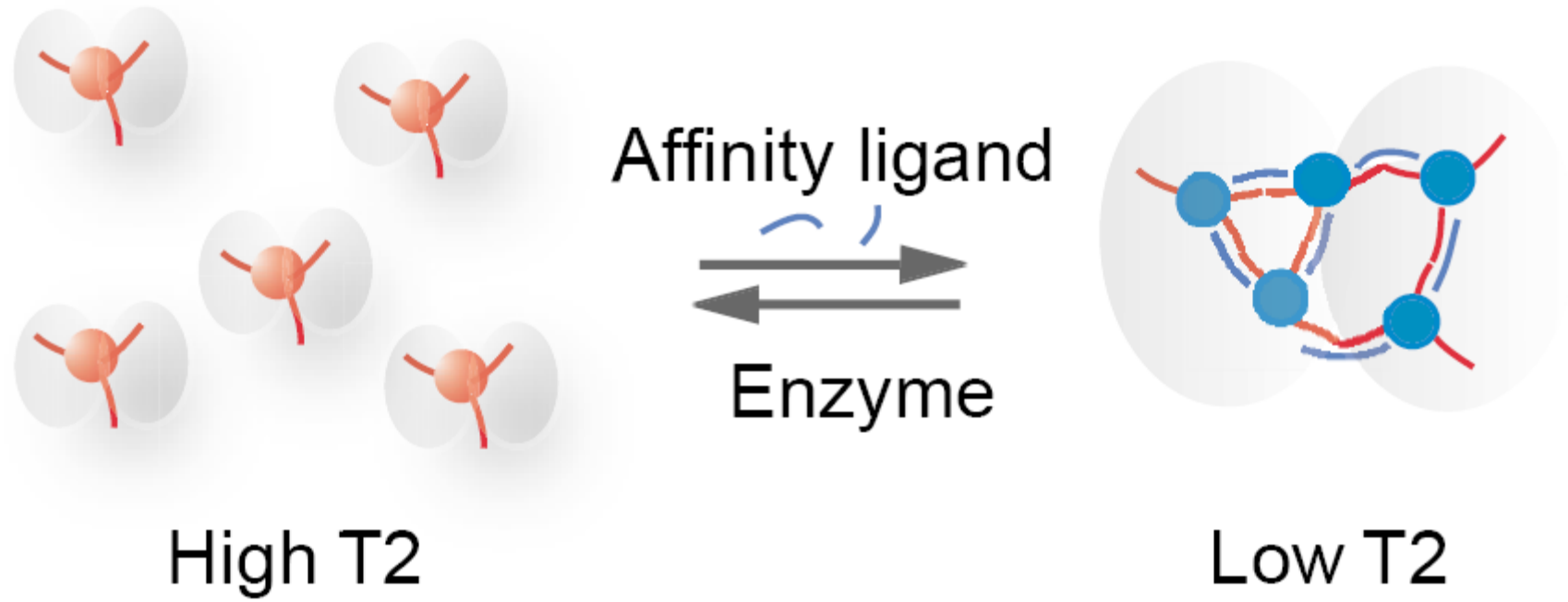
Lee Josephson, and Ralph Weissleder*

***Nature Biotech*. 2002, 20, 816.**

- Detect molecular interactions in the reversible self-assembly of disperse magnetic nanoparticles into stable assemblies
- 4 different molecular interactions: DNA-DNA, protein-protein, protein-small molecule, enzyme reactions
- MRI detectable in turbid media
- Potential in vivo imaging

Working principle of MRS

- During this cooperative process, SPIO core of individual nanoparticles becomes more efficient at dephasing the spins of surrounding water protons,
- Enhancing spin-spin (T2) relaxation times



DNA hybridization generates assembly of CLIO

→ Lowers T2

Solution color changes from red to blue upon the analyte-directed aggregation of gold nanoparticles, a consequence of interacting particle surface plasmons and aggregate scattering properties.

Melting profiles of the nanoparticle-labeled DNA aggregates were extraordinarily sharp, occurring over a temperature range much more narrow than the transition for unlabeled or conventional fluorophore-labeled DNA.

By virtue of sharp melting transitions target DNA could be differentiated from DNA with single base-pair mismatches simply by measuring absorbance (or looking at color) as a function of temperature.

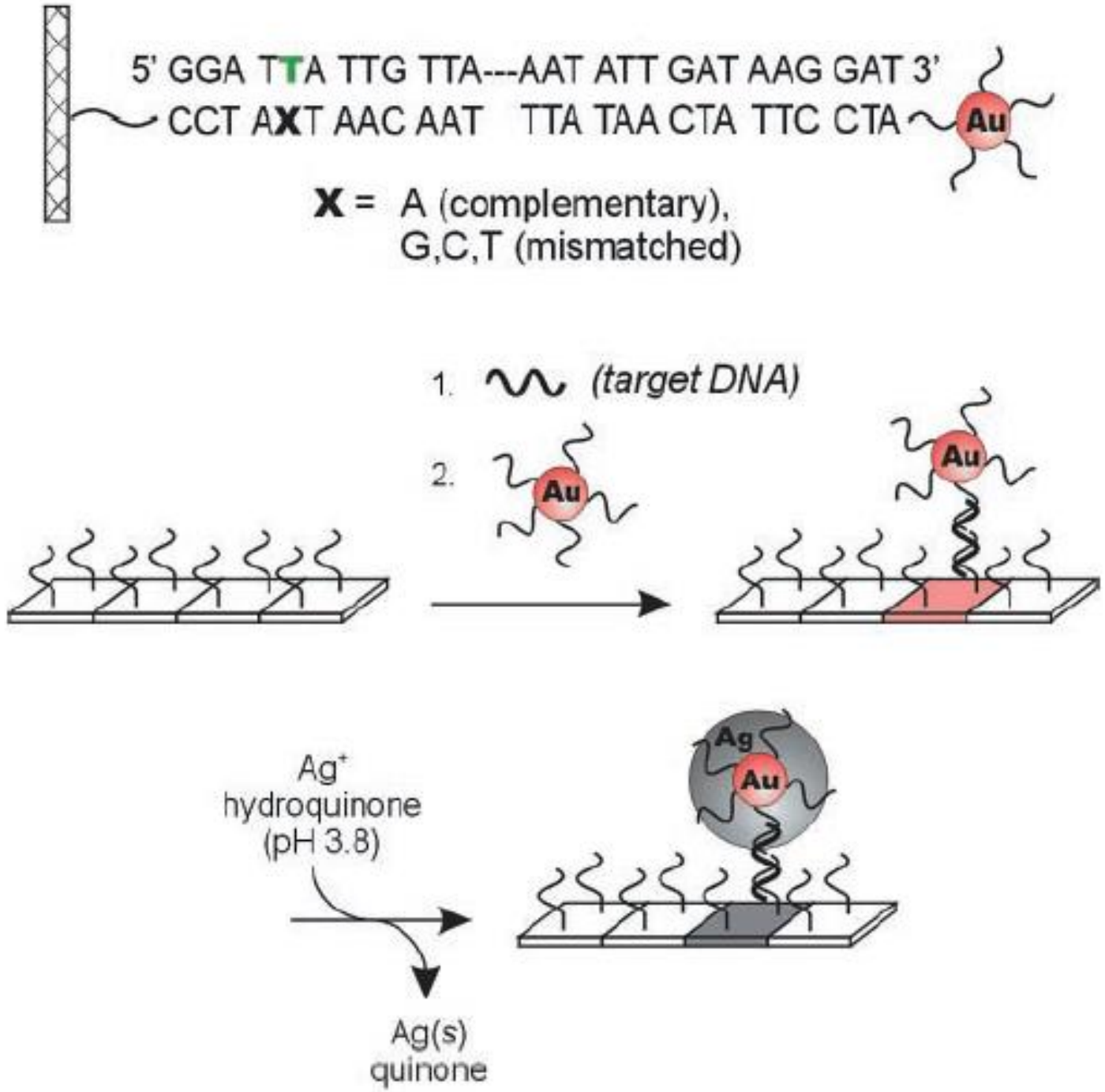
Scanometric DNA Array Detection with Nanoparticle Probes

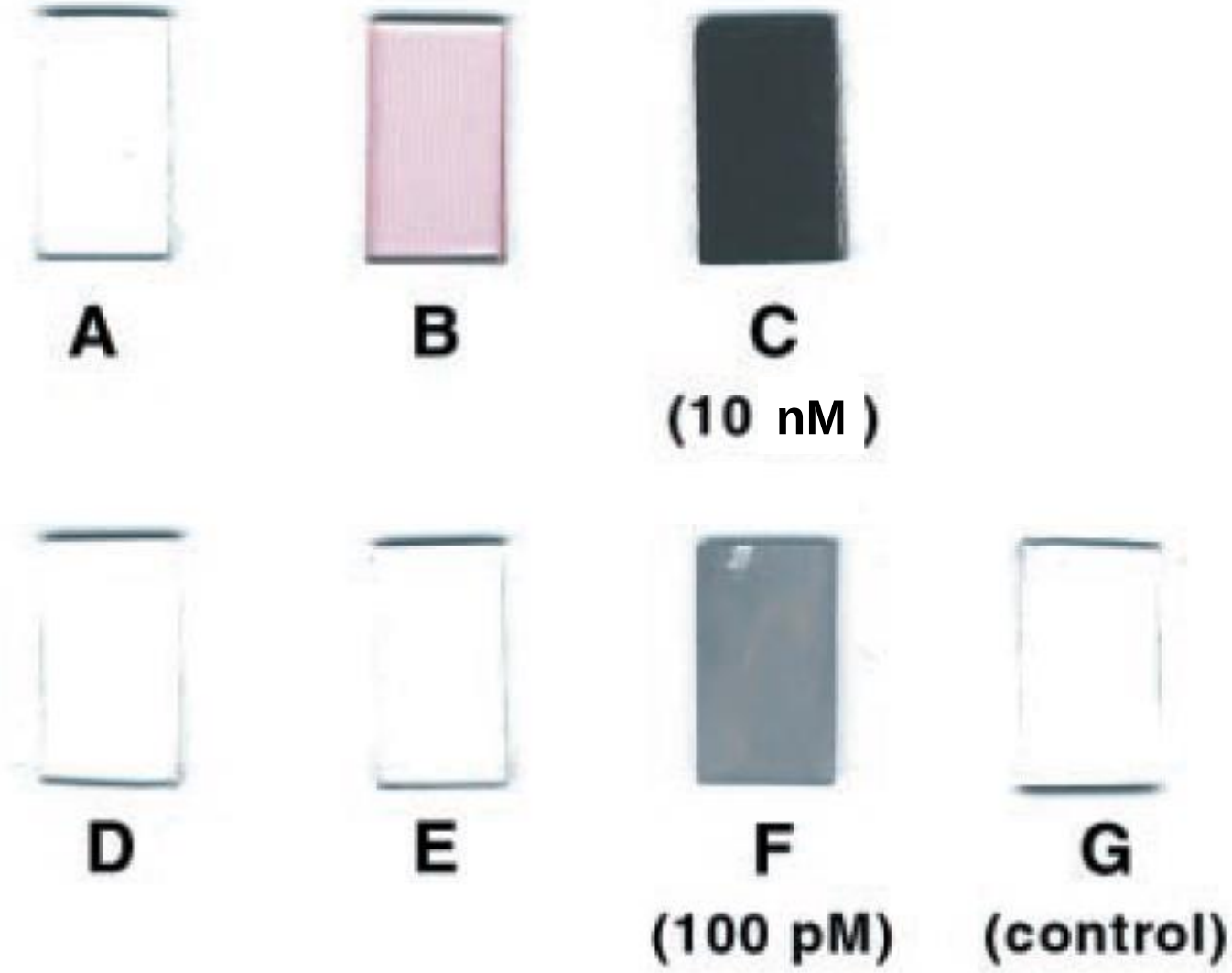
T. Andrew Taton,1,2 Chad A. Mirkin,1,2* Robert L. Letsinger1*

Science **2000**, 289, 1757.

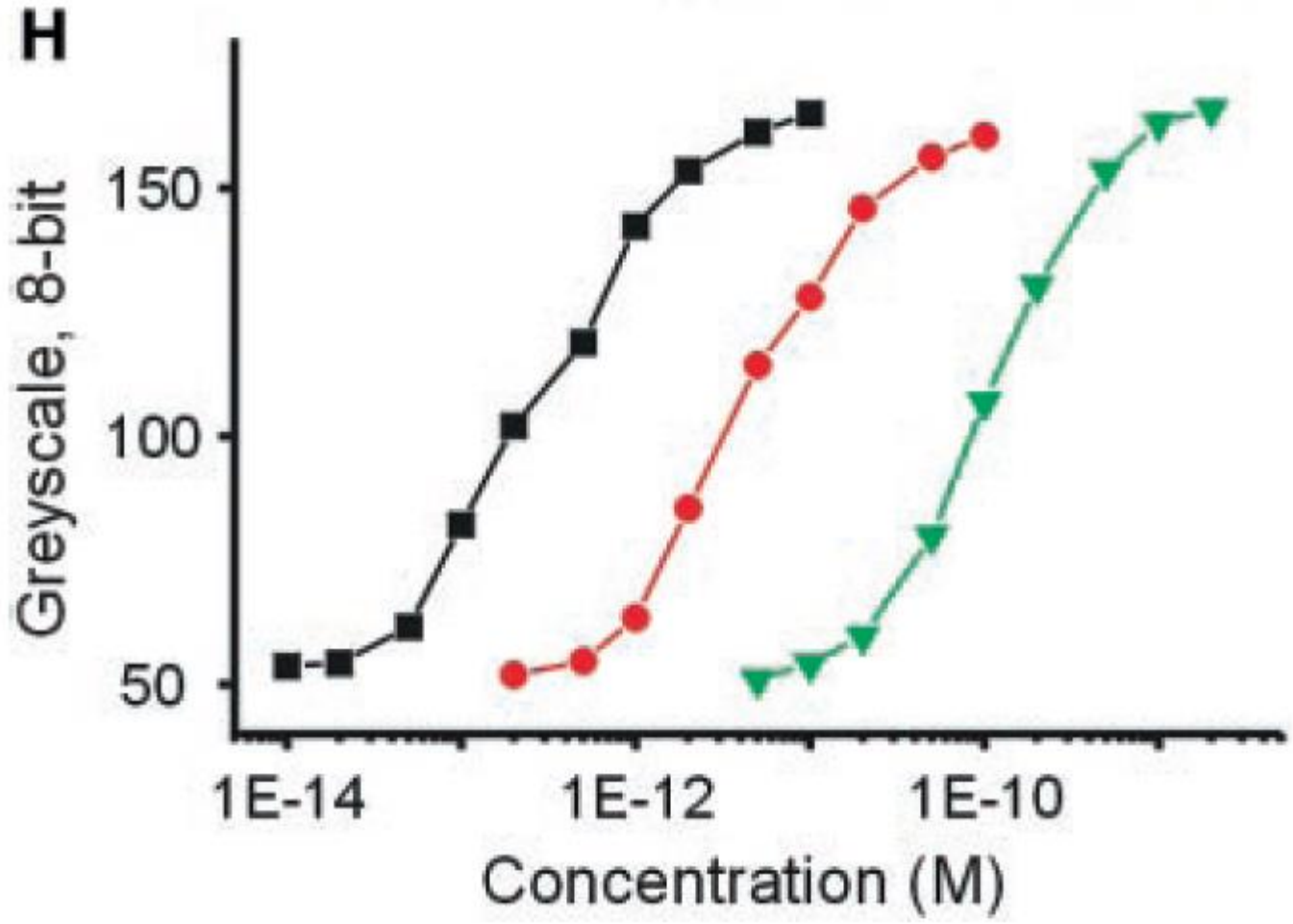
- Specific hybridization of surface-bound, single strand capture oligonucleotides to complementary targets.
- Both the specificity and sensitivity of these assays are dependent on the dissociation properties of capture strands hybridized to perfect and to mismatched complements.
- These network structures exhibit exceptionally sharp melting profiles; FWHM as low as 2°C.
- Sharp melting transitions allow one to differentiate a perfectly complementary target strand from a strand with a single base mismatch

• Analyzing combinatorial DNA arrays using oligonucleotide-modified gold nanoparticle probes
• Melting profiles of the targets from an array substrate.
• Discrimination of an oligonucleotide sequence from targets with single nucleotide mismatches with a selectivity that is over three times that observed for fluorophore-labeled targets.
• When coupled with a signal amplification method based on nanoparticle-promoted reduction of silver(I), the sensitivity of this scanometric array detection system exceeds that of the analogous fluorophore system by two orders of magnitude.

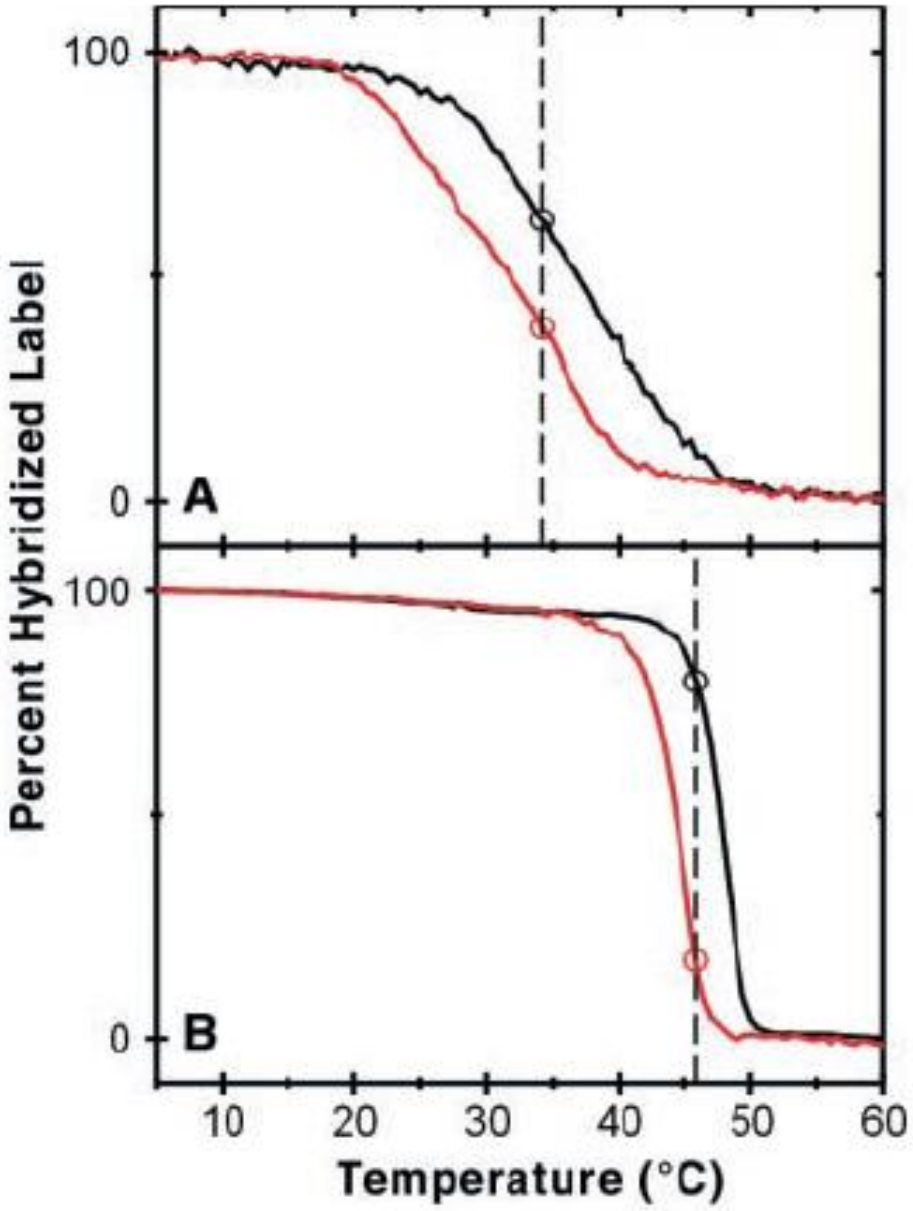

5' GGA TTA TTG TTA---AAT ATT GAT AAG GAT 3'
CCT AXT AAC AAT TTA TAA CTA TTC CTA
Au
X = A (complementary),
G,C,T (mismatched)
1.
(target DNA)
2.
Au
Au
Ag+
hydroquinone
(pH 3.8)
Ag
Au
Ag(s)
quinone


A
B
C
(10 nM)
D
E
F
(100 pM)
G
(control)

The lowest target concentration that can be effectively distinguished from the background baseline is 50 fM.

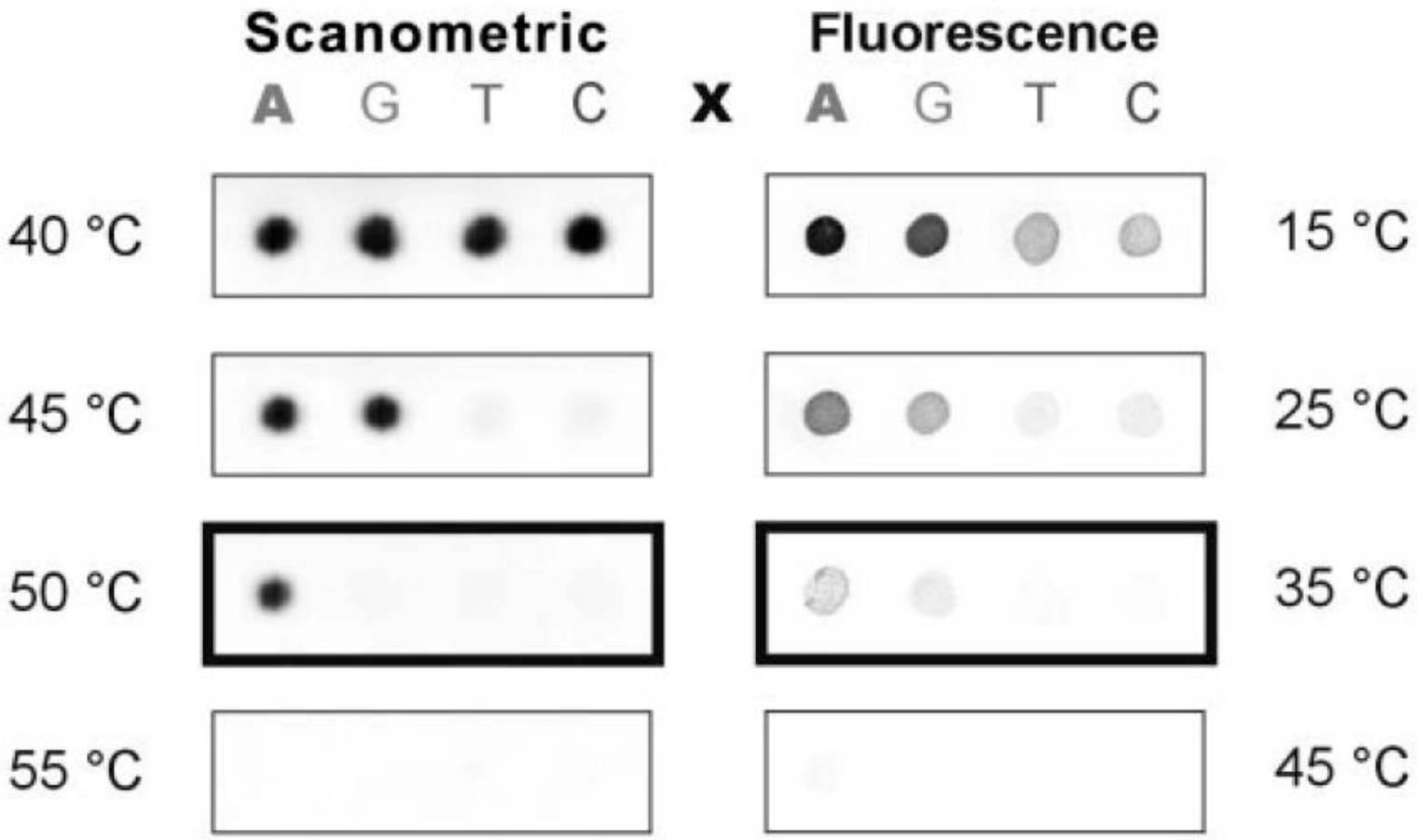


Melting curves for relative selectivity



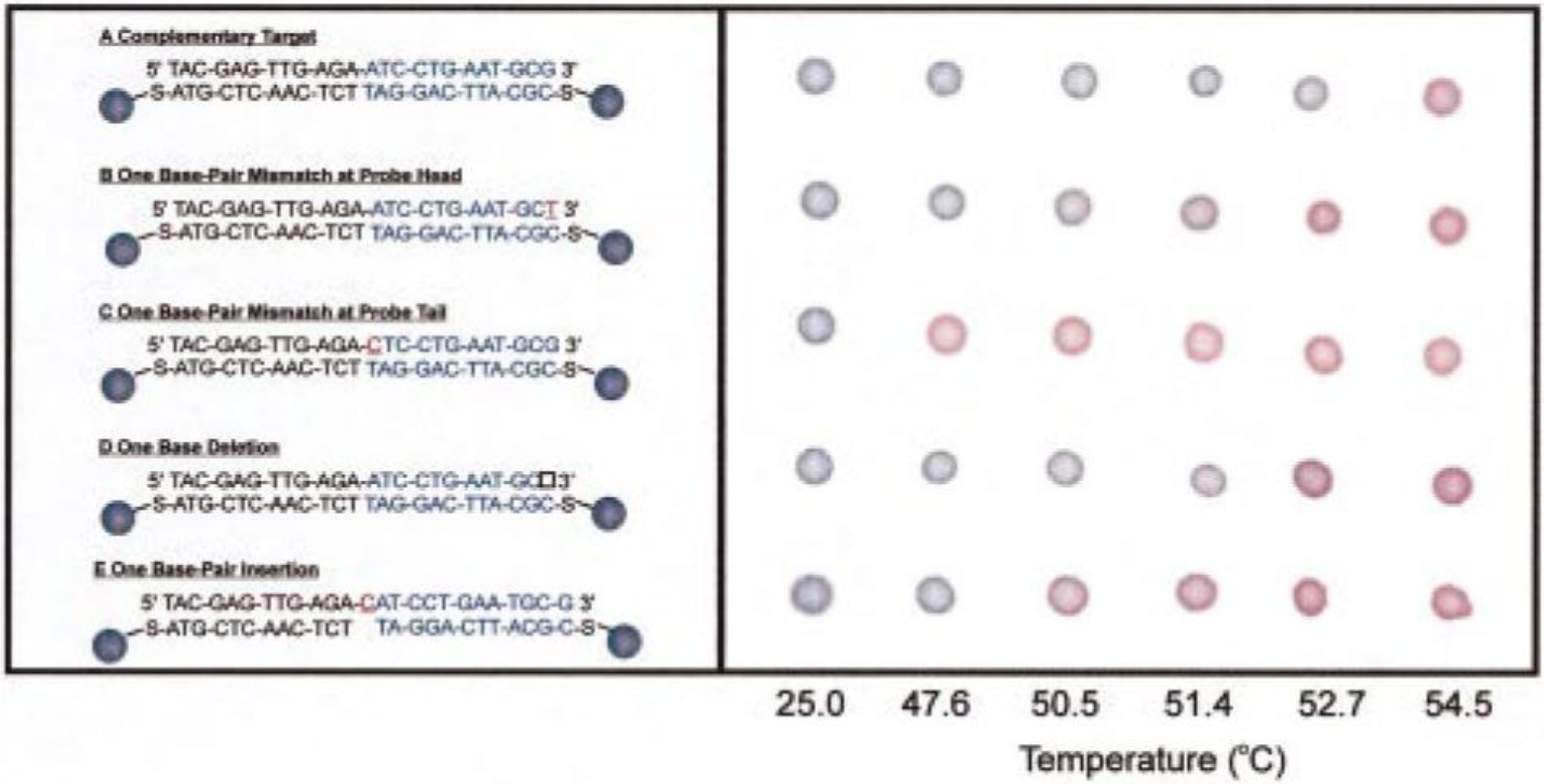
FWHM of 18 C

3 C



Hybridization signal could be resolved at the X 5 A elements at target concentrations as low as 50 fM (*5*);
this represents a 100-fold increase in sensitivity over that of Cy3-labeled arrays imaged by confocal fluorescence microscopy, for which target concentrations of > 5 pM required

“Northwestern Spot Test” for polynucleotide detection



VERIGENE SYSTEM

Nanosphere's Verigene® System enables clinicians to rapidly identify and treat the bacteria and viruses responsible for some of the most complex, costly and deadly infectious diseases.

The Verigene platform offers automated, cost-effective multiplex capabilities that rapidly and accurately detect infectious pathogens and drug resistance markers without relying on time-consuming culture methods.

Delivery of this time-critical information enables clinicians to provide targeted patient care more quickly, potentially leading to improved patient outcomes, lower costs, optimized antibiotic therapy, reduced spread of antibiotic resistance and importantly, saved lives.

Verigene is powering faster treatment decisions in more than half of the top US hospitals. Verigene's ease of use makes it a valuable system for use in both hospital-based and reference laboratories, regardless of size, and features:

Nanosphere's tests are designed to target infections of the bloodstream, respiratory tract and gastrointestinal tract. The technology has also demonstrated capabilities for cardiovascular disease, cancer and autoimmune disease.

On-demand workflow (24/7 testing)

Load Test Cartridge, test consumables, and sample into Processor *SP*

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Automated sample preparation and test processing on Processor *SP*

Minimal hands-on time

CLIA moderate complexity

Nanosphere is dedicated to enhancing medicine by providing targeted molecular diagnostic tests that can lead to earlier disease detection, optimal patient treatment and improved healthcare economics.

Our platform, the Verigene® System, enables clinicians to rapidly identify and treat the bacteria and viruses responsible for some of the most complex, costly and deadly infectious diseases.
Headquartered in Northbrook, Illinois, Nanosphere was founded in 1999 based upon technology developed at Northwestern University by Dr. Robert Letsinger and Dr. Chad Mirkin. In 2007, Nanosphere went public (NASDAQ: NSPH), and has remained committed to continued scientific discovery and innovation with more than 175 patents to its name.
Currently, our test menu targets infections of the bloodstream, respiratory tract and gastrointestinal tract.