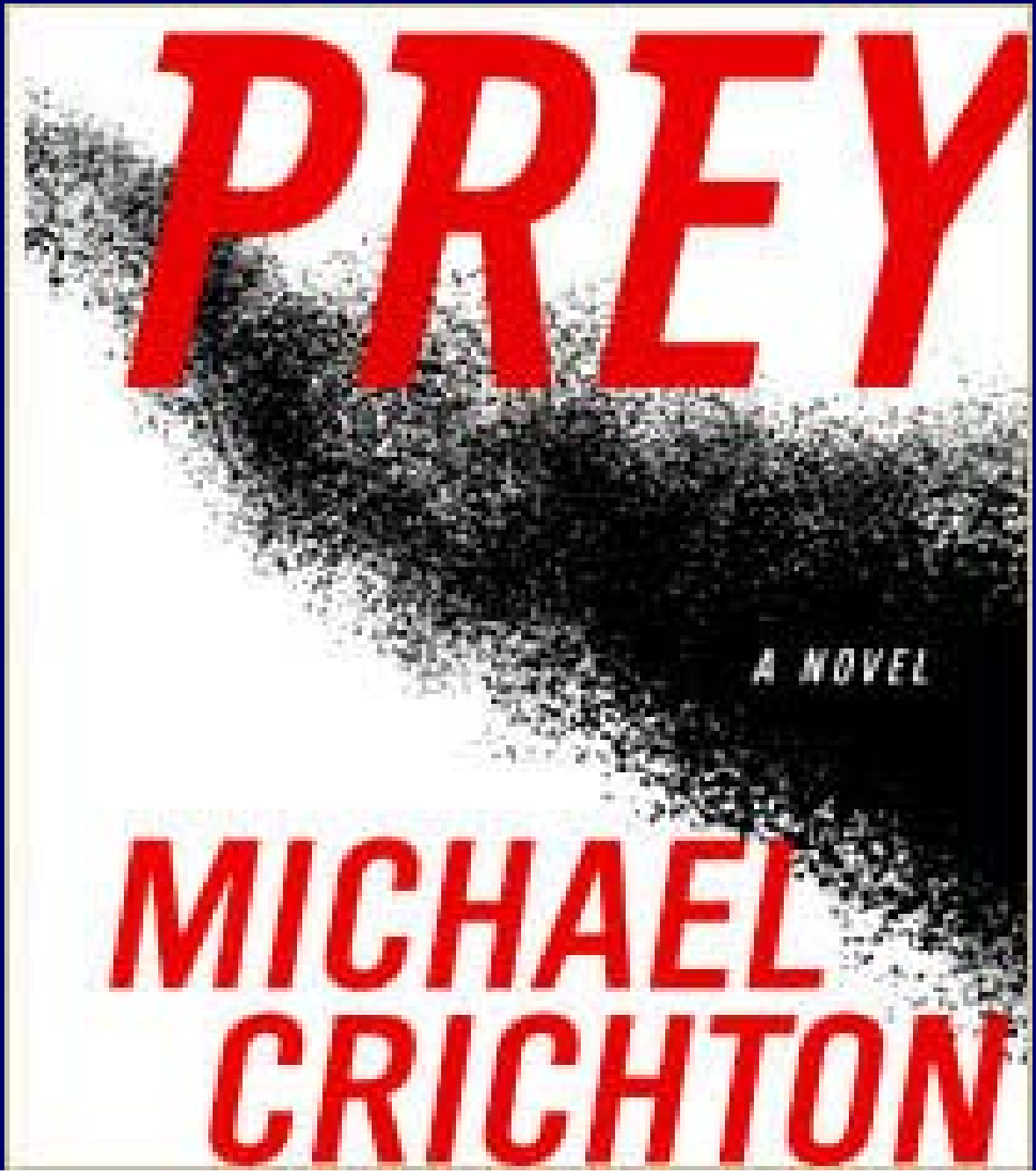
Nanotoxicology Update

Don Fisher, MD, MS, DABT

1 April 2008


PREY
A NOVEL
MICHAEL
CRICHTON

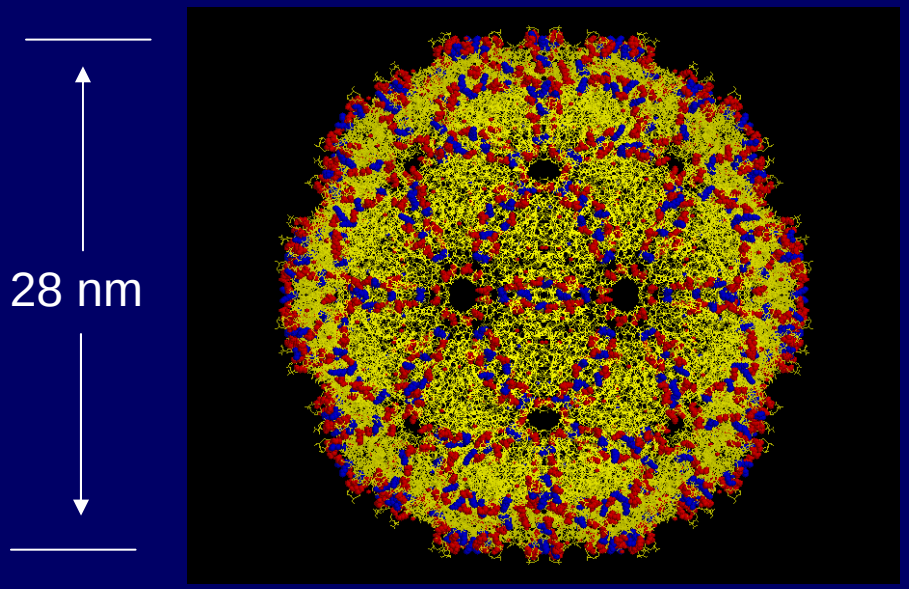
Model 2D system: self-assembly of viral particles

-Viruses are naturally monodisperse, and have surfaces that can be modified using genetic engineering

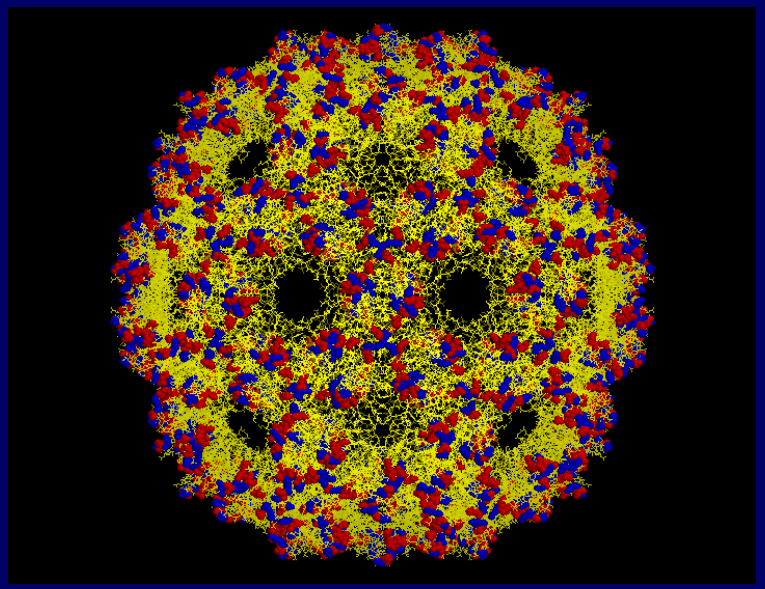
-In addition to use as a model system for simulations of self-assembly, we hope to use viral arrays as scaffolds for nanoparticle organization

C. Ashley, D Peabody, D. Dunphy…

MS2



Q-Beta



Control charge and distribution Red = positive charge, blue = negative charge

Characterizing the little buggers

- At least 1 dimension of 1 to 100 nm
- Particles – fumes, smokes, exhausts
- Balls – fullerenes
- Tubes – single and multi-walled
- Dendrimers – branching spheres (spherical snowflakes)
- Quantum dots – packaged crystals

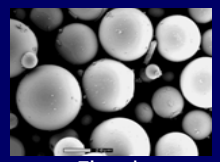
The Scale of Things – Nanometers and More

Things Natural

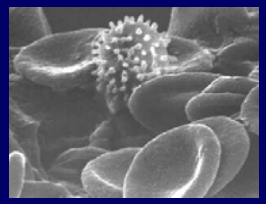
Dust mite
200 μm

Ant
~ 5 mm

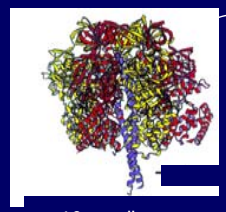
Human hair
~ 60-120 μm wide



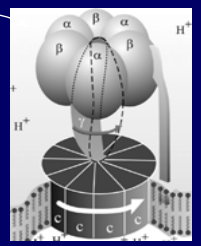
Fly ash
~ 10-20 μm



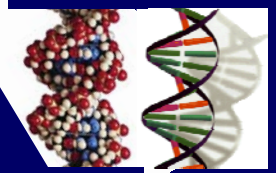
Red blood cells
with white cell
~ 2-5 μm



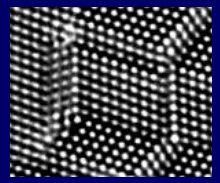
~10 nm diameter



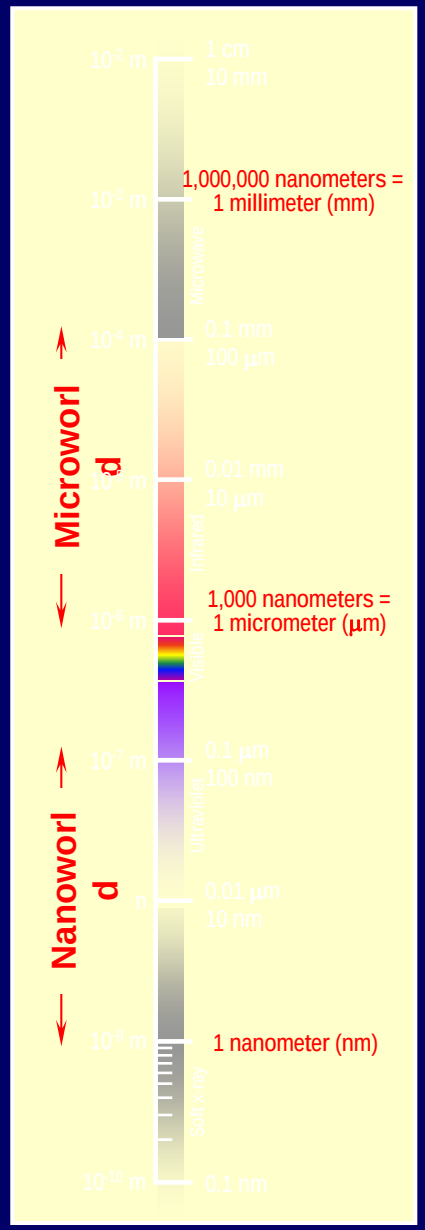
ATP synthase



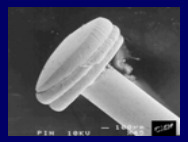
DNA
~2-1/2 nm diameter



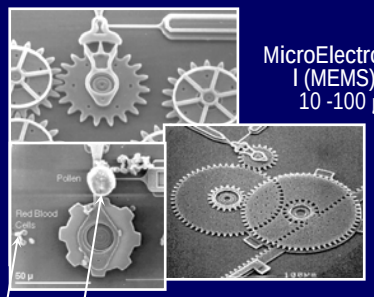
Atoms of silicon
spacing ~tenths of nm



Things Manmade

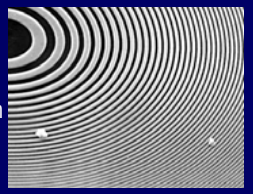


Head of a pin
1-2 mm

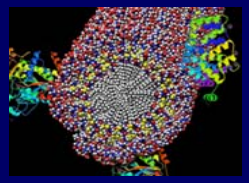


MicroElectroMechanical (MEMS) devices
10 -100 μm wide

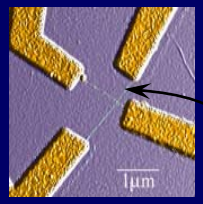
Pollen grain
Red blood cells



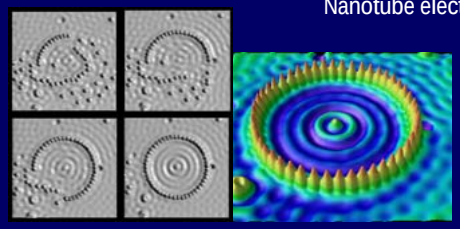
Zone plate x-ray "lens"
Outer ring spacing ~35 nm



Self-assembled,
Nature-inspired structure
Many 10s of nm

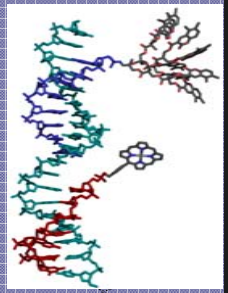


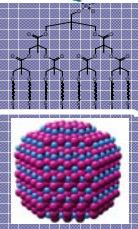
Nanotube electrode



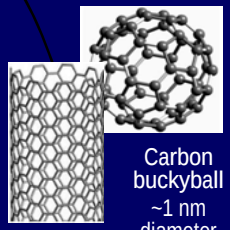
Quantum corral of 48 iron atoms on copper surface
positioned one at a time with an STM tip
Corral diameter 14 nm

The Challenge





Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.



Carbon buckyball
~1 nm diameter

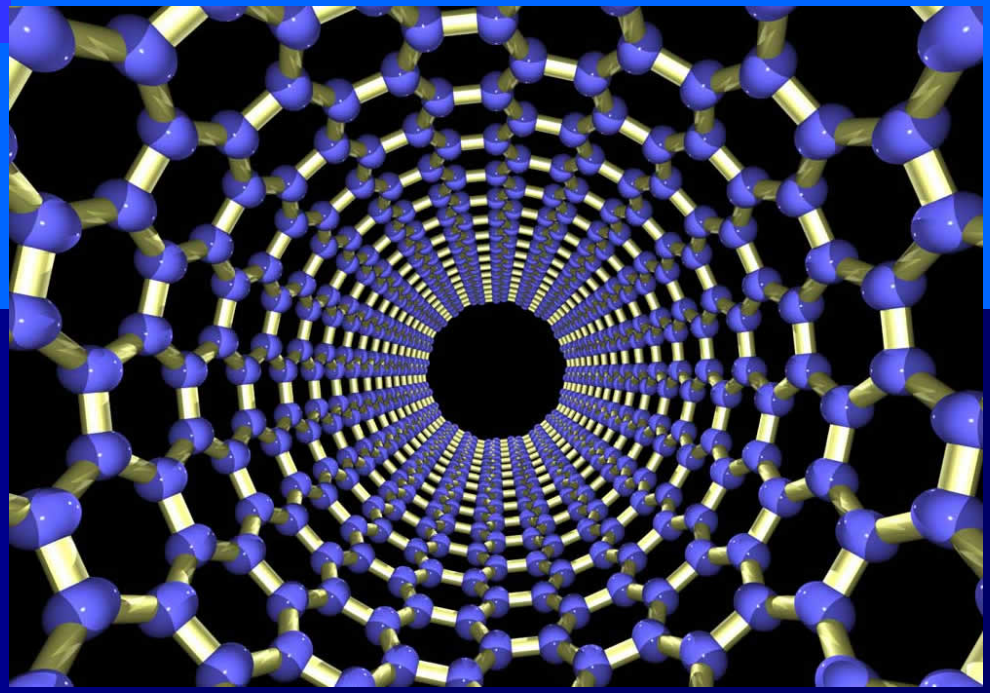
Carbon nanotube
~1.3 nm diameter

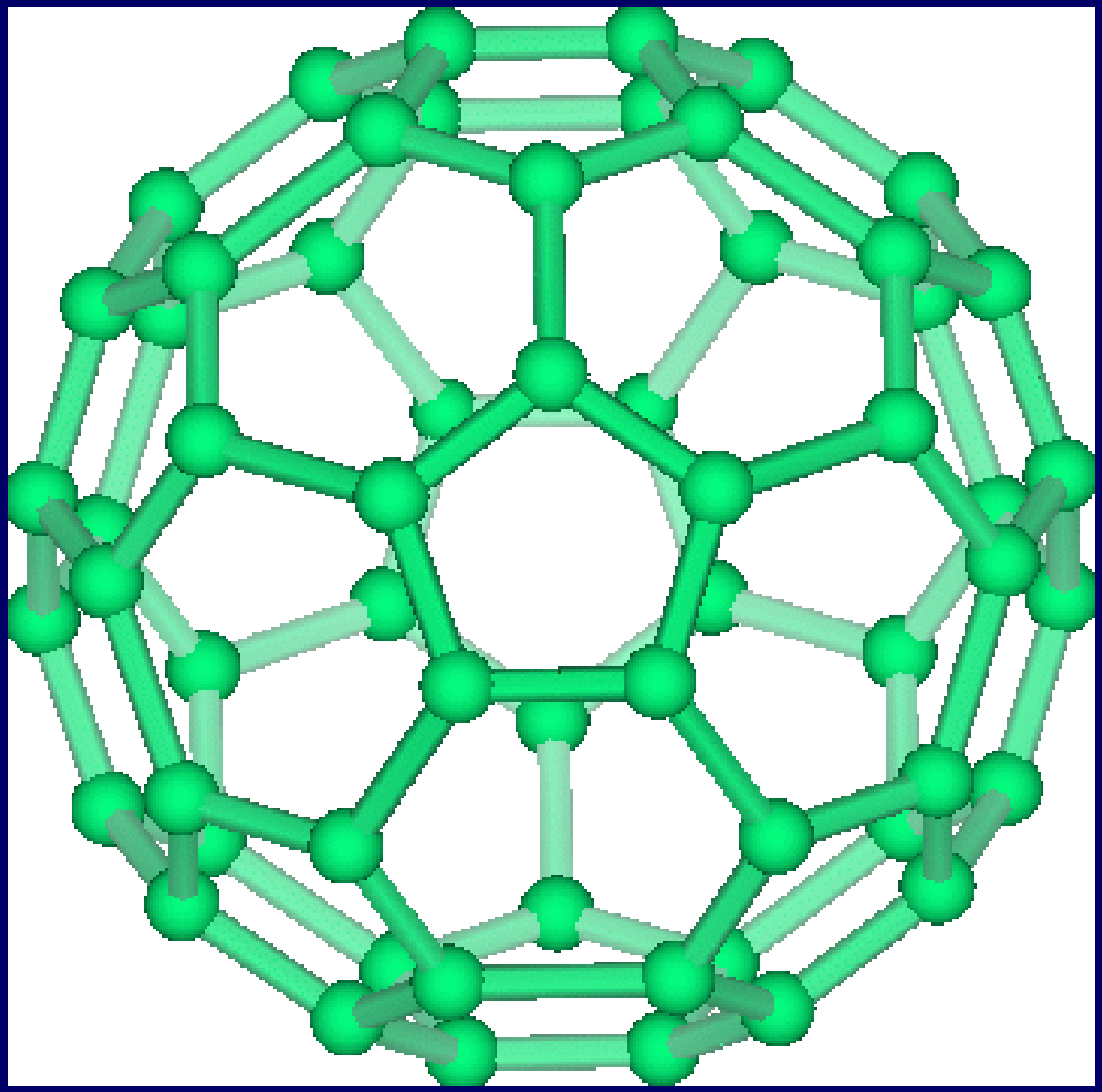
Office of Basic Energy Sciences
Office of Science, U.S. DOE
Version 10-07-03, pmd

Products

- Fullerenes – discovered in 1985. Nobel Prize in 1996
- Carbon nanotubes – discovered 1991 by Iijima
  - Single walled (SWCNT)
  - Multi-walled (MWCNT) – 2 or more layers
- Quantum dots (Qdots)
  - Fluorescent
  - “Functional” coatings
- Semiconductors

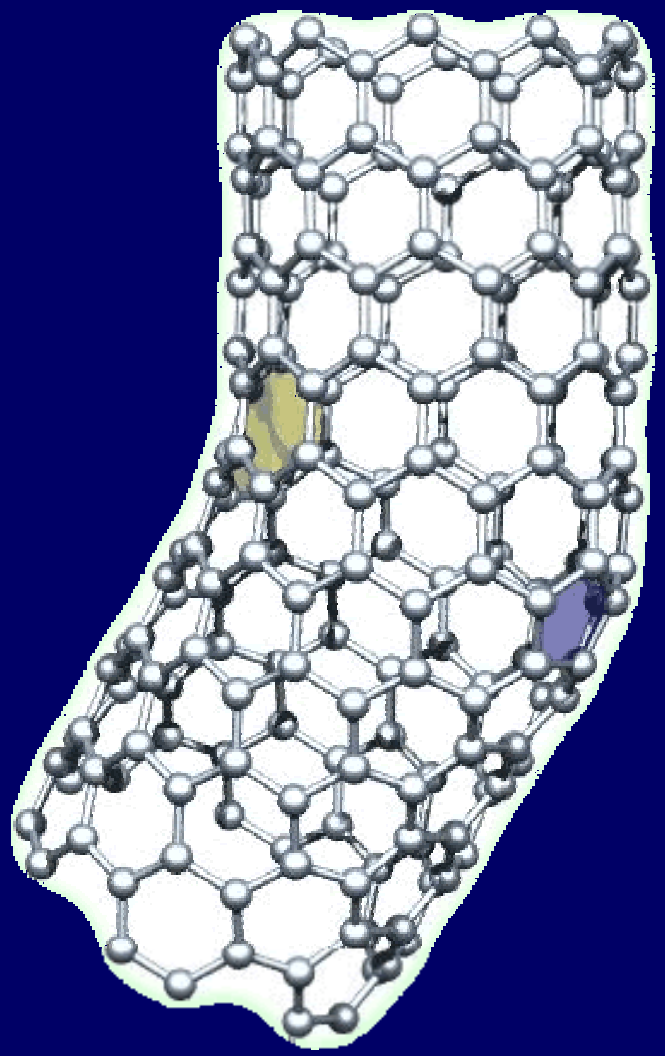
Fullerenes & Carbon Nanotubes

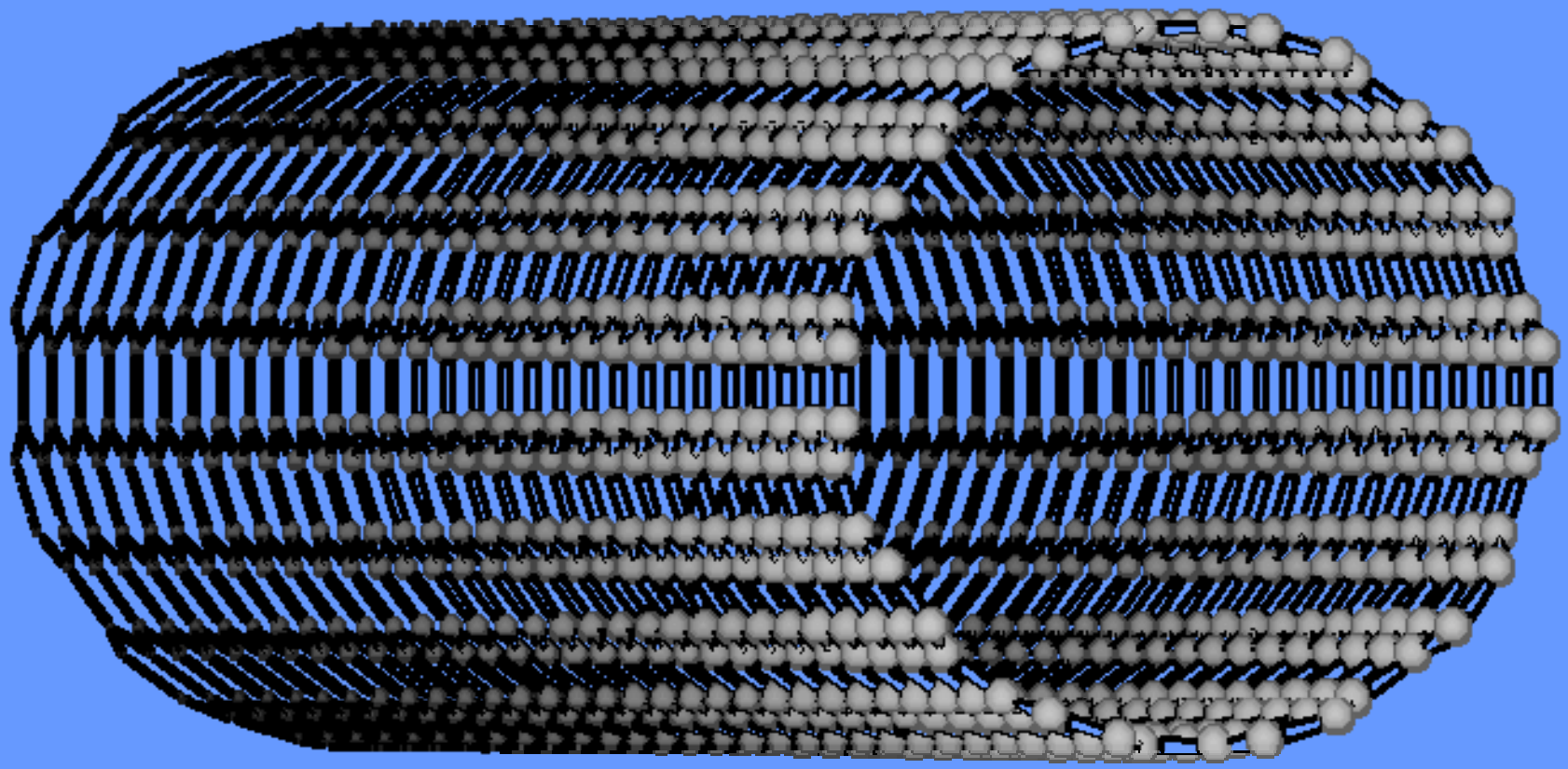




Special Properties

- Difference in chemical reactivity for
  end caps and side wall
- High axial mechanical strength
- Special electrical properties:
  - Metallic
  - Semi conducting





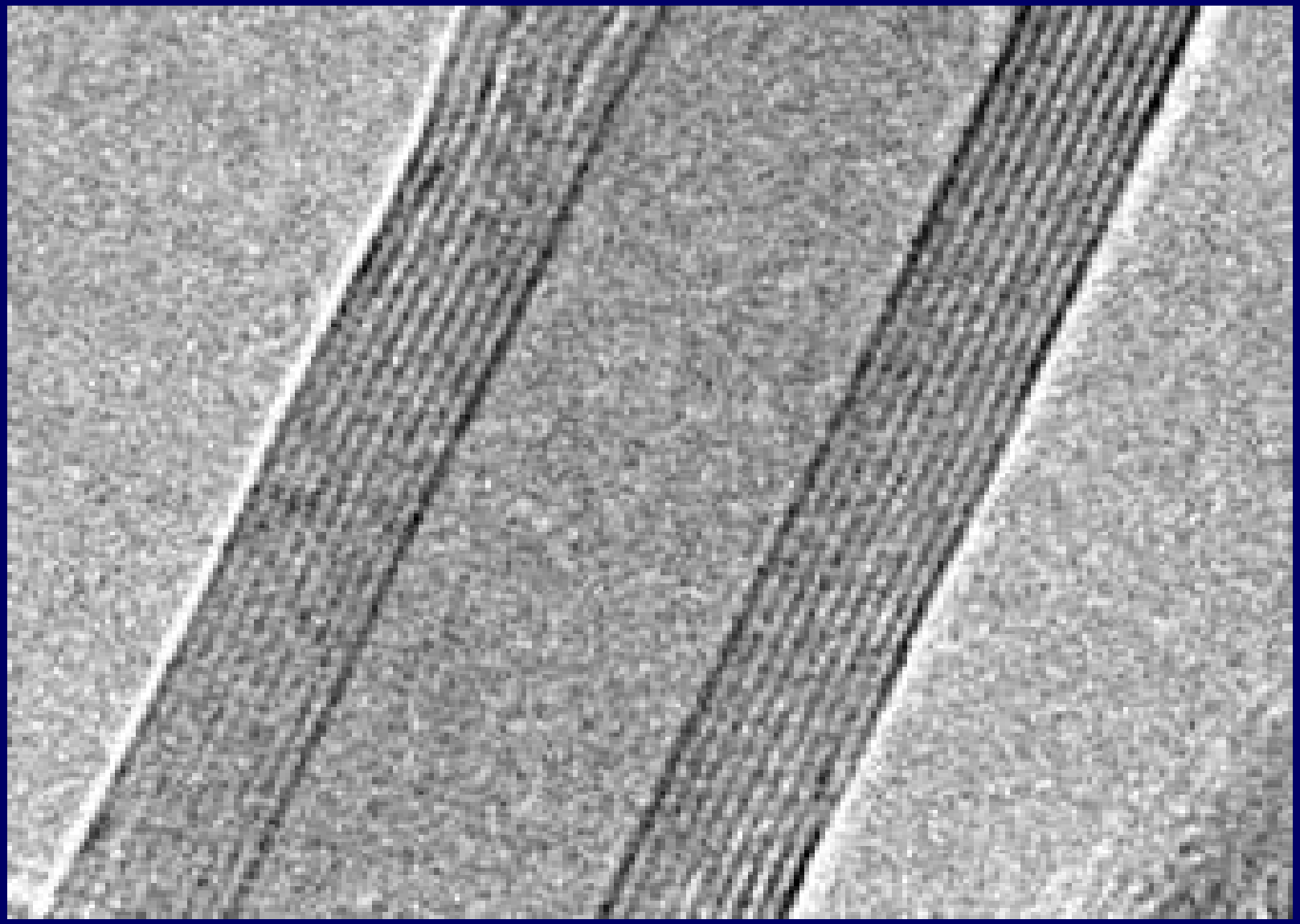
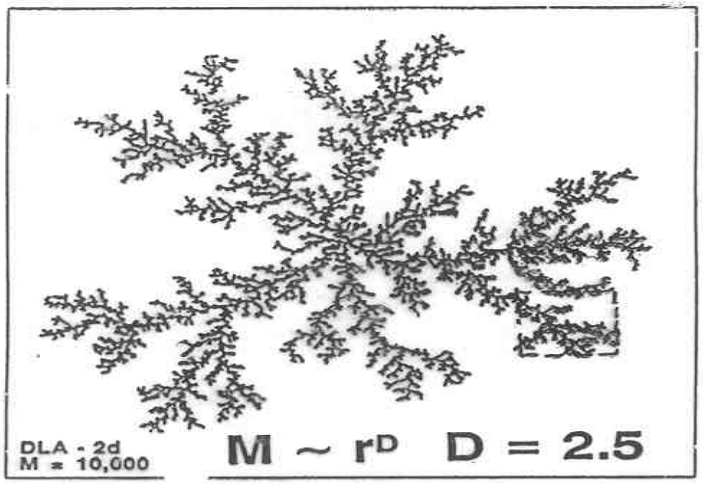


Image credit NanoLab Inc.

POROSITY OF HIERARCHICAL STRUCTURES

Porosity of Primary Cluster



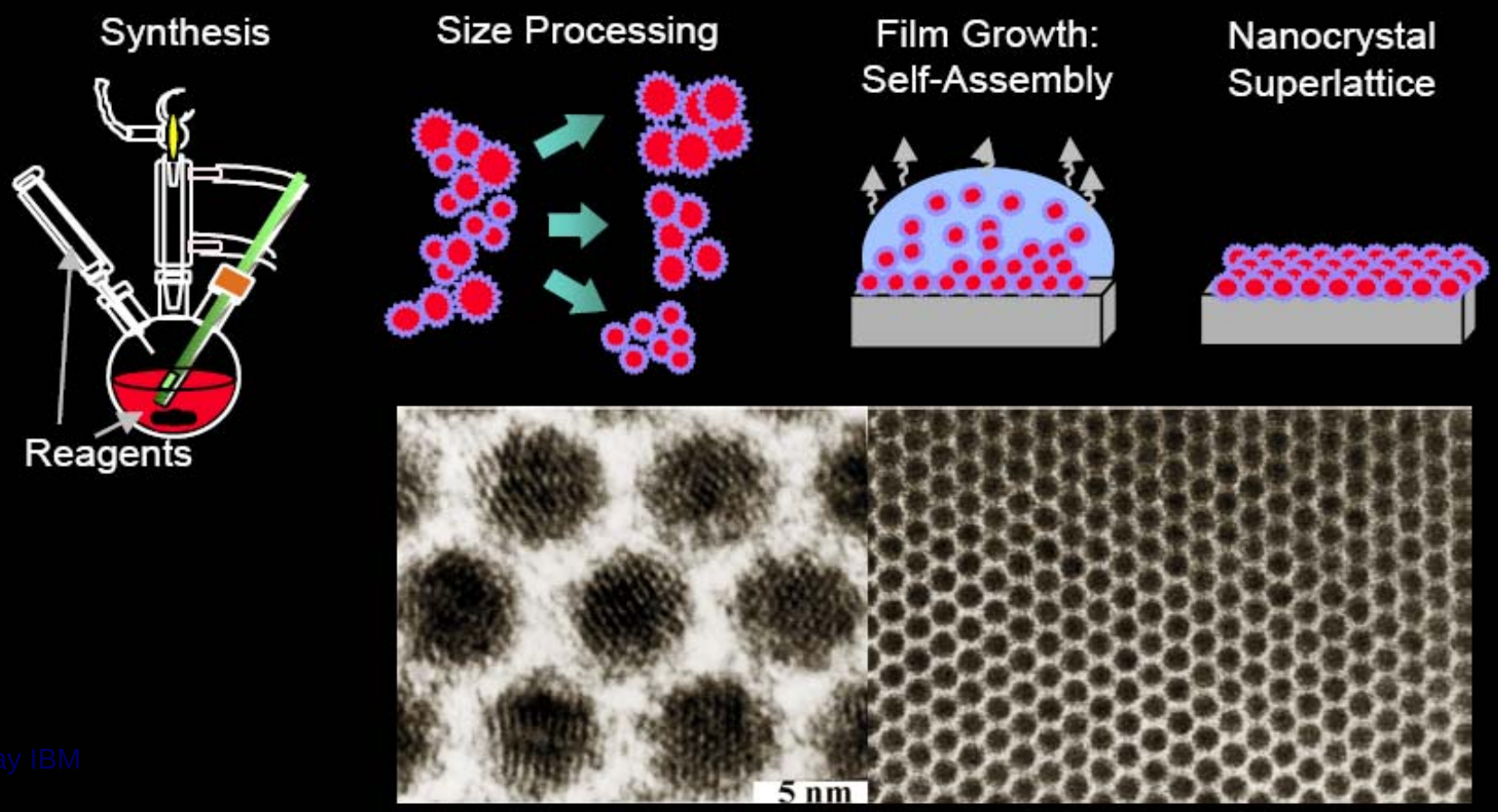
$$\text{Mass} = M \propto r^D, \; D \leq 3$$

$$\text{Density} = \text{Mass/Volume} = r^D/r^d = 1/r^{(3-D)}$$

$$\text{Porosity} \propto 1/\text{Density} = r^{(3-D)}$$

Sol-gel chemistry provides a means to tune fractal dimension (roughness Factor) and cluster size (volume fraction porosity) enabling systematic Control of wetting

“Guiding” or “directing” the assembly process:
Semiconductor Nanocrystals



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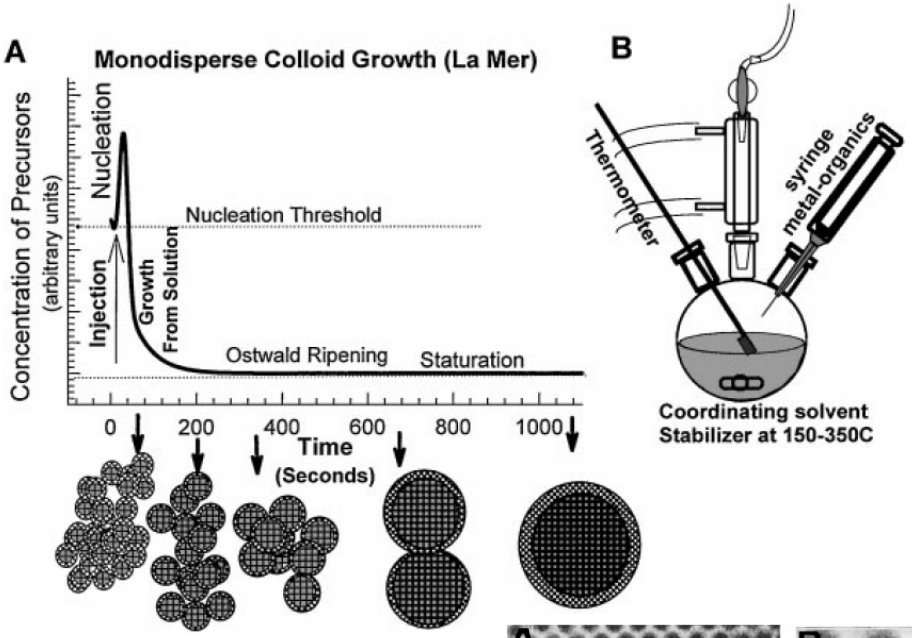
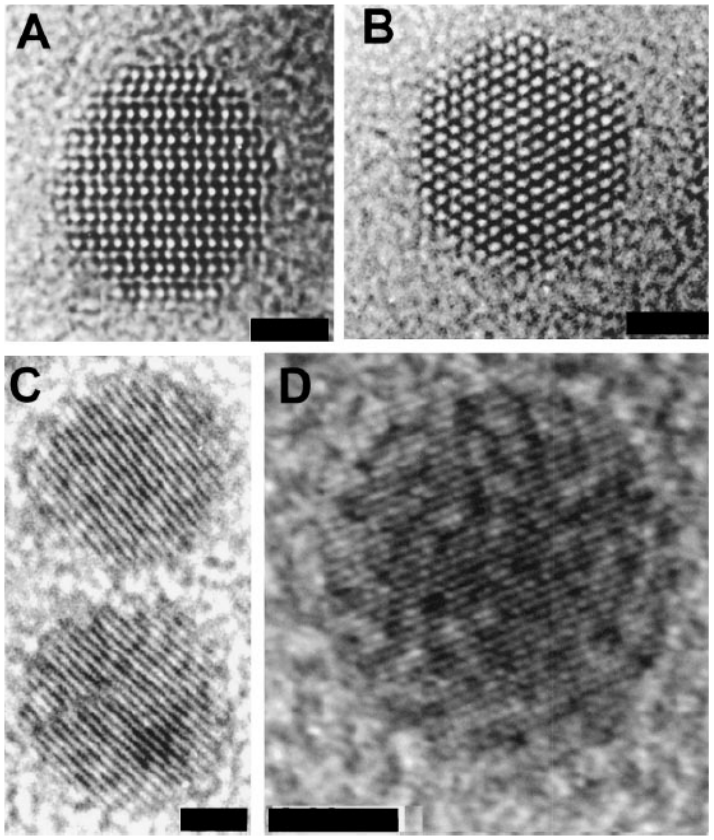
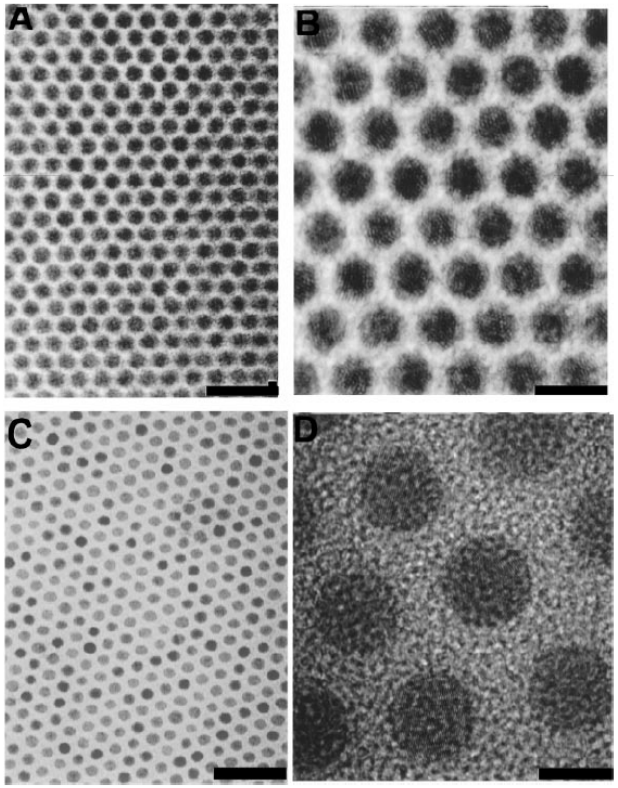
Pretty pictures but is this a sufficeintly robust material

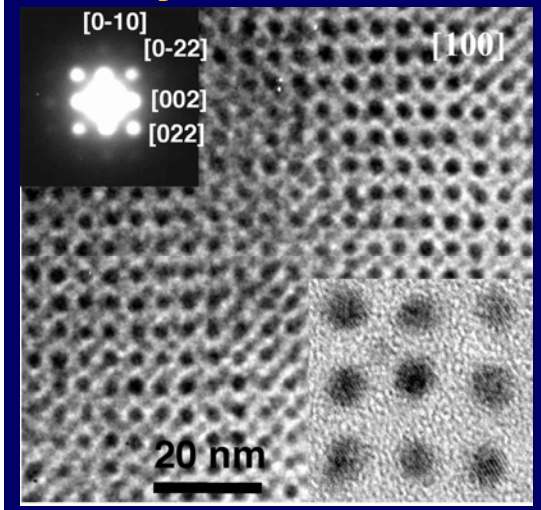

A
Monodisperse Colloid Growth (La Mer)
Concentration of Precursors
(arbitrary units)
Nucleation
Nucleation Threshold
Injection
Growth
From Solution
Ostwald Ripening
Staturation
0
200
400
600
800
1000
Time
(Seconds)
B
Thermometer
syringe
metal-organics
Coordinating solvent
Stabilizer at 150-350C


A
B
C
D

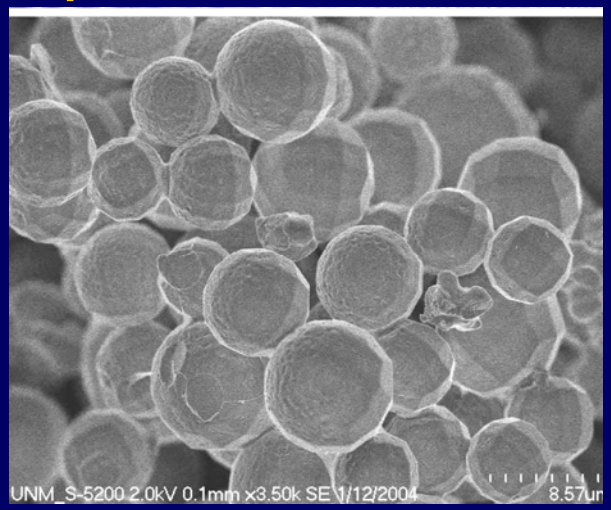

A
B
C
D

CJ Brinker

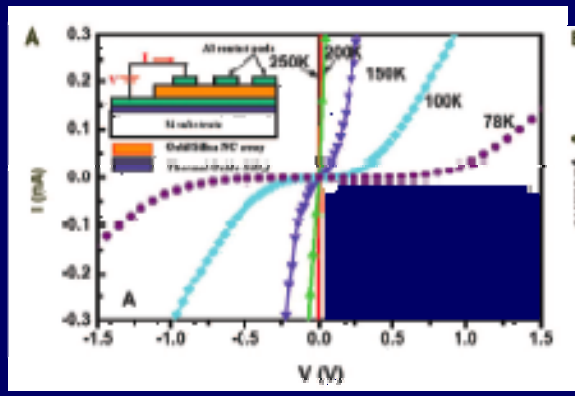
Self-Assembly and integration of robust 3D nanocrystal arrays - sensors, plasmonics, electronics

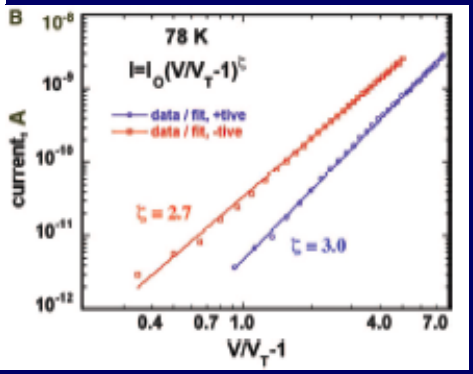


25˚C, TEOS, gold, CTAB



0˚C, 4 days, TEOS, gold, CTAB





Self-Assembly of Ordered, Robust, Three-Dimensional Gold Nanocrystal/Silica Arrays

Hongyou Fan,[1,2*] Kai Yang,[2] Daniel M. Boye,[4] Thomas Sigmon,[3] Kevin J. Malloy,[3] Huifang Xu,[2] Gabriel P. López,[2] C. Jeffrey Brinker[1,2*]

We report the synthesis of a new nanocrystal (NC) mesophase through self-assembly of water-soluble NC micelles with soluble silica. The mesophase comprises gold nanocrystals arranged within a silica matrix in a face-centered cubic lattice with cell dimensions that are adjustable through control of the nanocrystal diameter and/or the alkane chain lengths of the primary alkanethiol stabilizing ligands or the surrounding secondary surfactants. Under kinetically controlled silica polymerization conditions, evaporation drives self-assembly of NC micelles into ordered NC/silica thin-film mesophases during spin coating. The intermediate NC micelles are water soluble and of interest for biolabeling. Initial experiments on a metal-insulator-metal capacitor fabricated with an ordered three-dimensional gold nanocrystal/silica array as the "insulator" demonstrated collective Coulomb blockade behavior below 100 kelvin and established the current-voltage scaling relationship for a well-defined three-dimensional array of Coulomb islands.

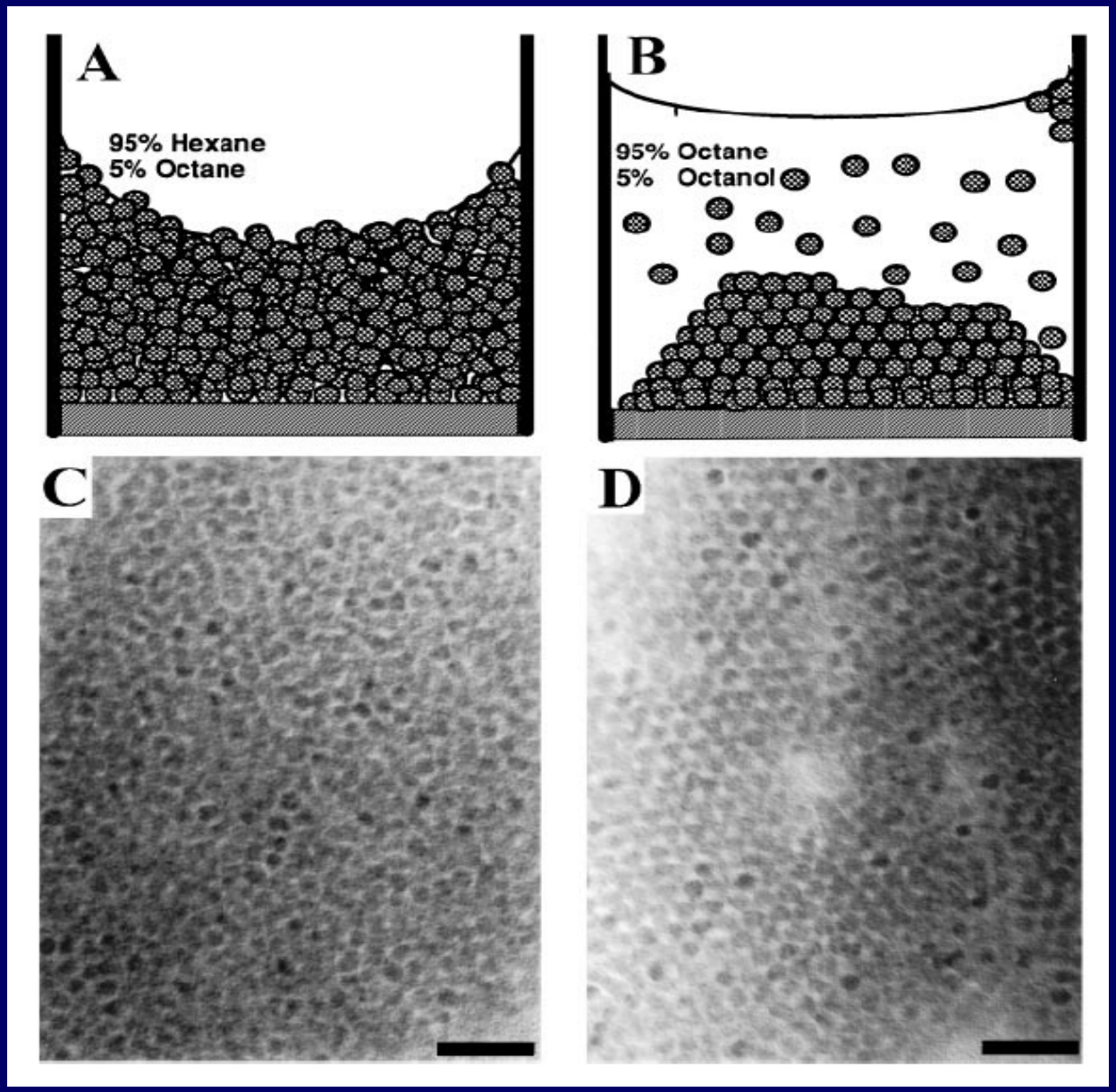
Despite recent advances in the synthesis and characterization of nanocrystals and NC arrays (*1*, *2*), there remain numerous challenges that limit their practical use. First, for example, synthesis procedures generally used for metallic and semiconducting NCs use organic passivating ligands that make the NCs water insoluble. This is problematic for biological imaging and more generally for uniform incorporation of nanocrystals in hydrophilic matrices like silica or titania needed for the fabrication of robust, functional lasers (*3*, *4*). Second, while steric stabilization of nanocrystals with organic passivating layers suppresses attractive particle-particle interactions, thereby facilitating self-assembly of NC arrays, it necessarily causes the arrays to be mechanically weak and often thermally and chemically unstable. Third, although evaporation of NC dispersions has been used to prepare quasi-3D NC arrays (*5*) and films containing isolated 3D NC islands (*6*), there exist no procedures to reliably fabricate 3D NC arrays as uniform thin films. These combined factors ultimately limit routine integration of nanocrystals into 3D artificial solid devices, in which electronic, magnetic, and optical properties could be tuned through electron charging and quantum confinement of individual NCs mediated by coupling interactions with neighboring NCs (*7*, *8*).

Here, we describe the direct synthesis of water-soluble NC gold micelles and their further self-assembly with silica into robust, ordered 3D NC arrays in bulk or thin-film forms. The synthetic approach is general and avoids the complicated multistep procedures reported previously (*9*). Our concept is to consider monosized, organically passivated NCs as large hydrophobic molecules that, if incorporated individually into the hydrophobic interiors of surfactant micelles, would result in the formation of monosized NC micelles composed of a metallic (or other) NC core and a hybrid bilayer shell with precisely defined primary and secondary layer thicknesses (Fig. 1H). The hydrophilic NC

[1]Sandia National Laboratories, Chemical Synthesis and Nanomaterials Department, Advanced Materials Laboratory, 1001 University Boulevard SE, Albuquerque, NM 87106, USA. [2]The University of New Mexico/NSF Center for Micro-Engineered Materials, Department of Chemical and Nuclear Engineering, and [3]Center for High Technology Materials, Albuquerque, NM 87131, USA. [4]Physics Department, Davidson College, Davidson, NC 28035, USA.

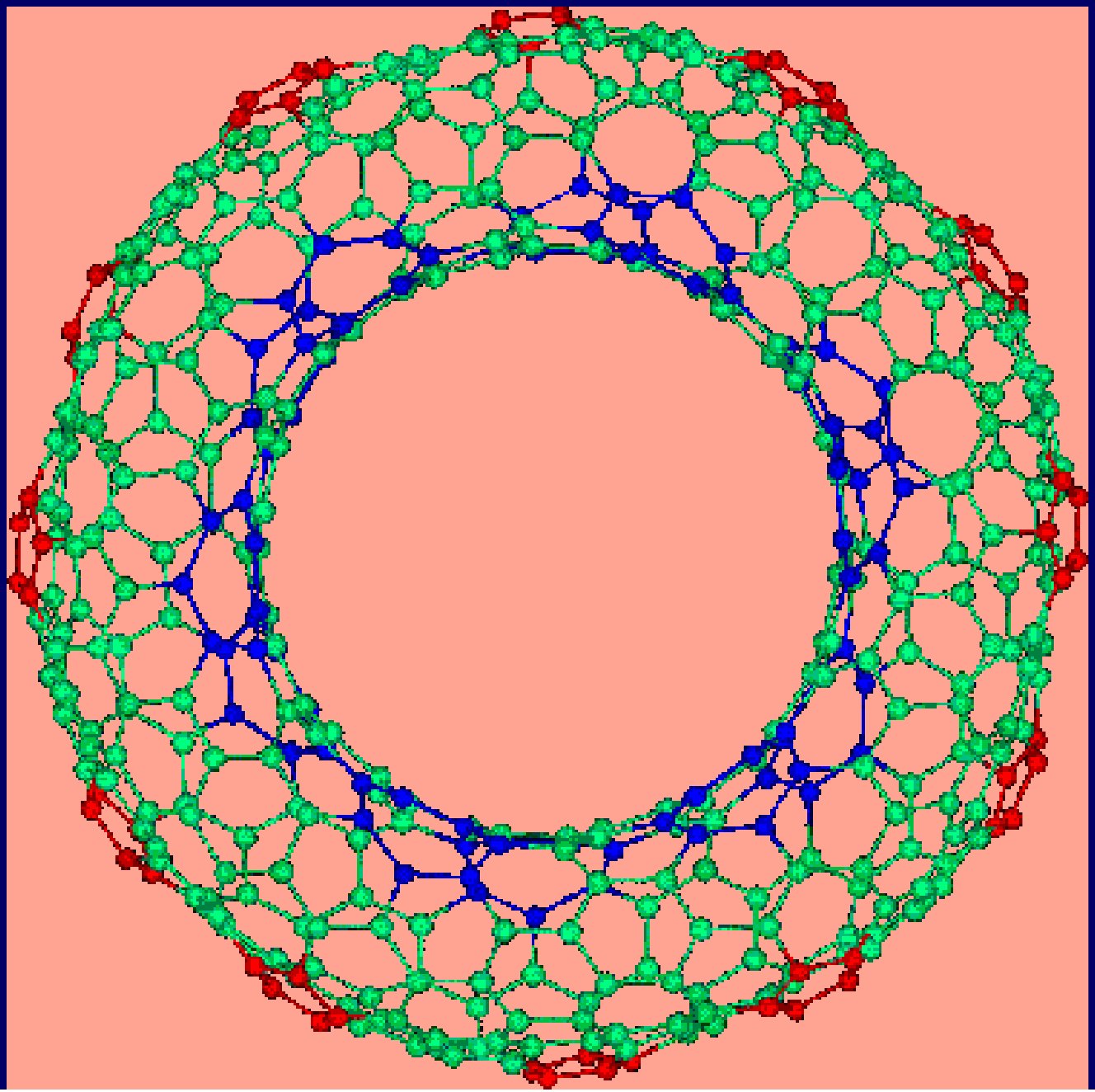
*To whom correspondence should be addressed. E-mail: hfan@sandia.gov (H.F.), cjbrink@sandia.gov (C.J.B.)

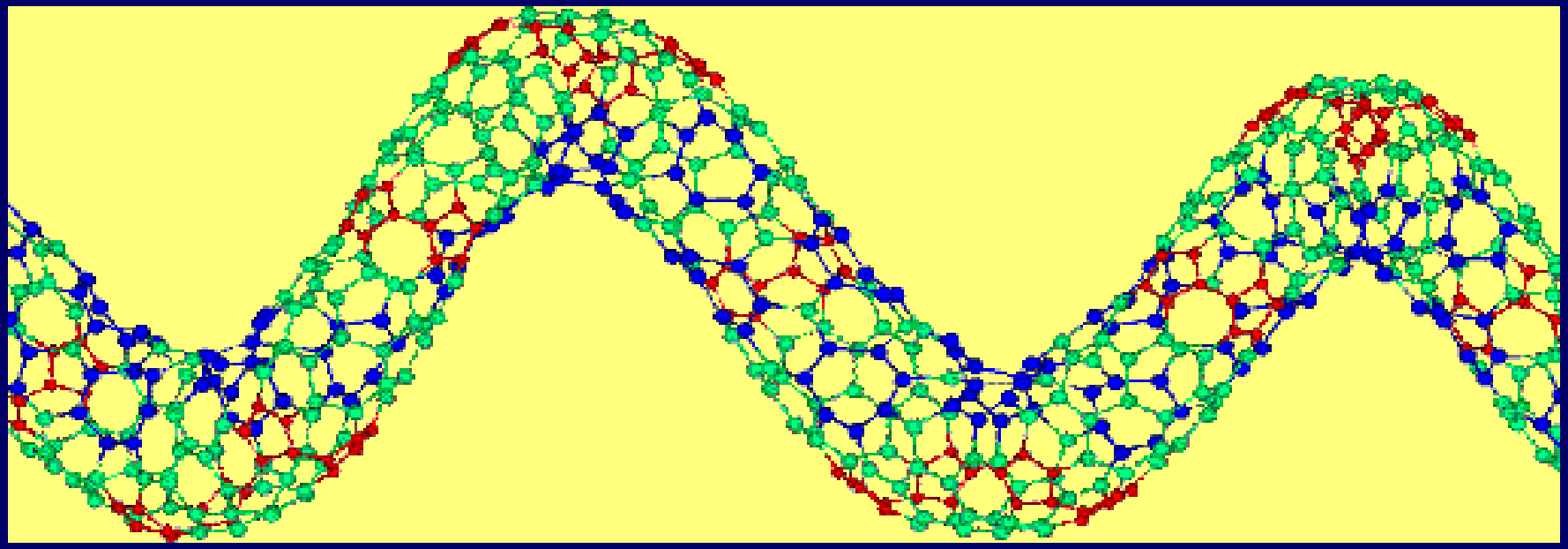
Fan *et al. Science* **304** 567 (2004). Model artificial solids to understand collective phenomena


A
95% Hexane
5% Octane
B
95% Octane
5% Octanol
C
D

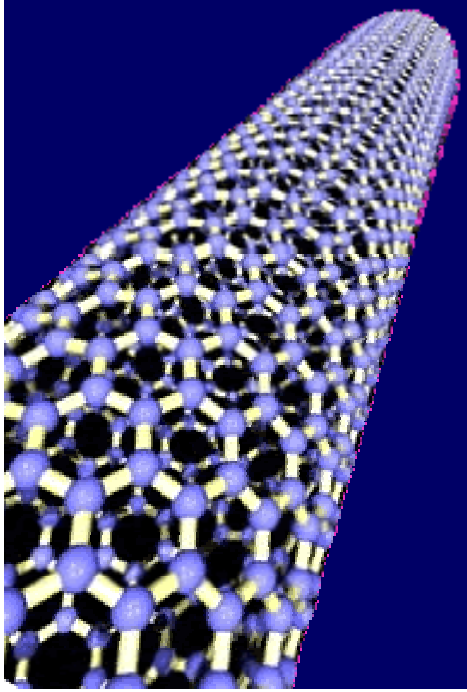
Historical Note

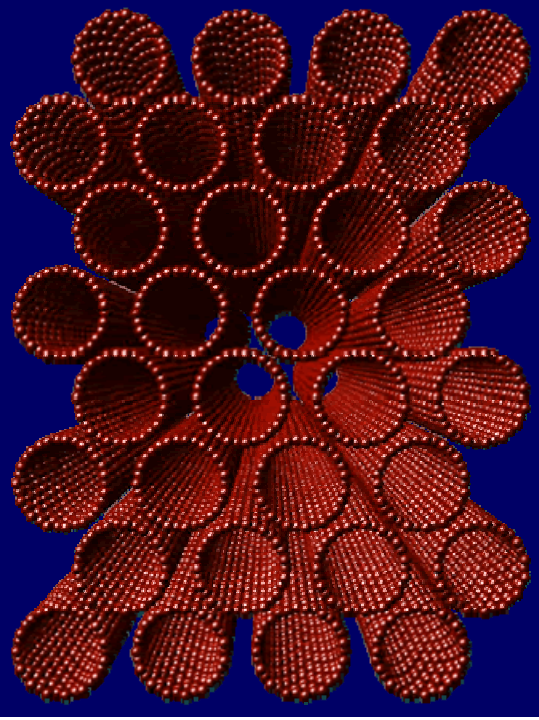
- 1959: Richard Feynman - precision machines at the atomic level possible
- 1970's: Eric Dressler – "molecular machines" to control manufacturing. Taniguchi – "nanotechnology"
- Late 90's - Federal funding for nanotech research
- 2000: concerns about safety

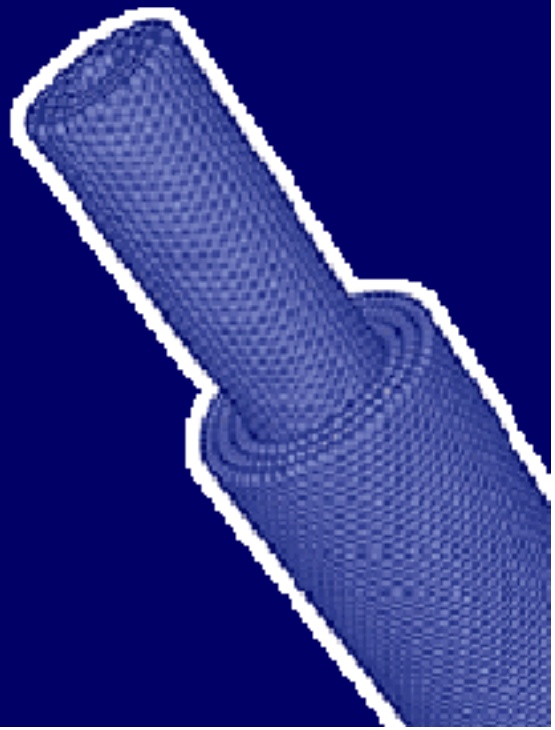




Commercial Applications



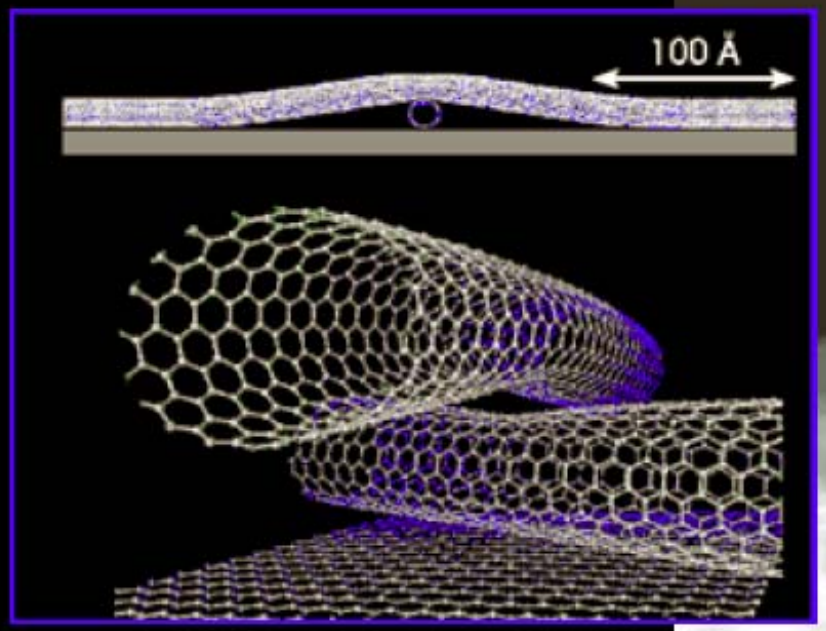


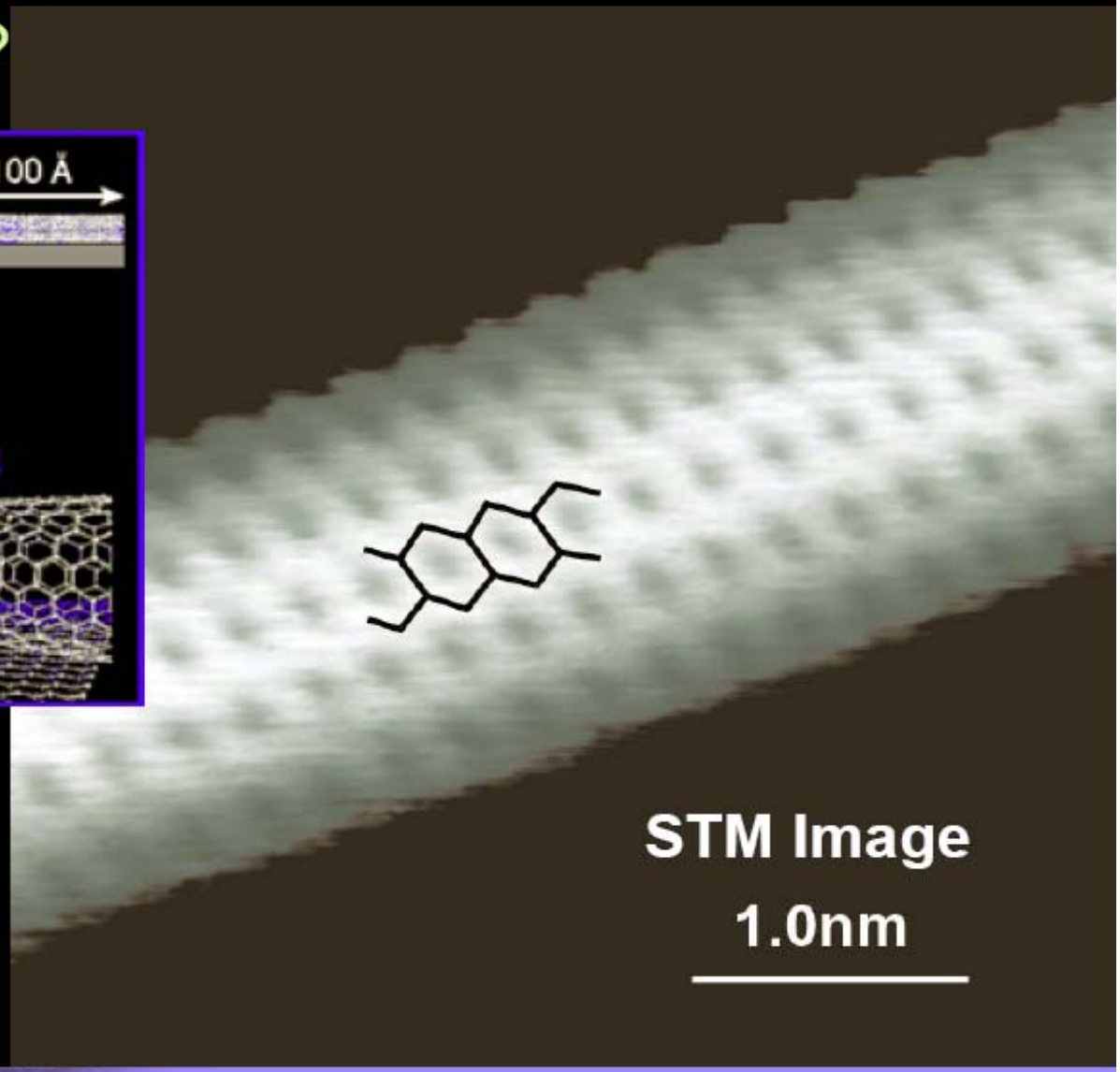


Potential Commercial Uses

- Spacecraft, space elevators
- Artificial muscles, combat jackets
- Land and sea vehicles
- Electrical conduction, power cables
- Semiconductors
- Drug delivery, medical imaging

Carbon Nanotubes?





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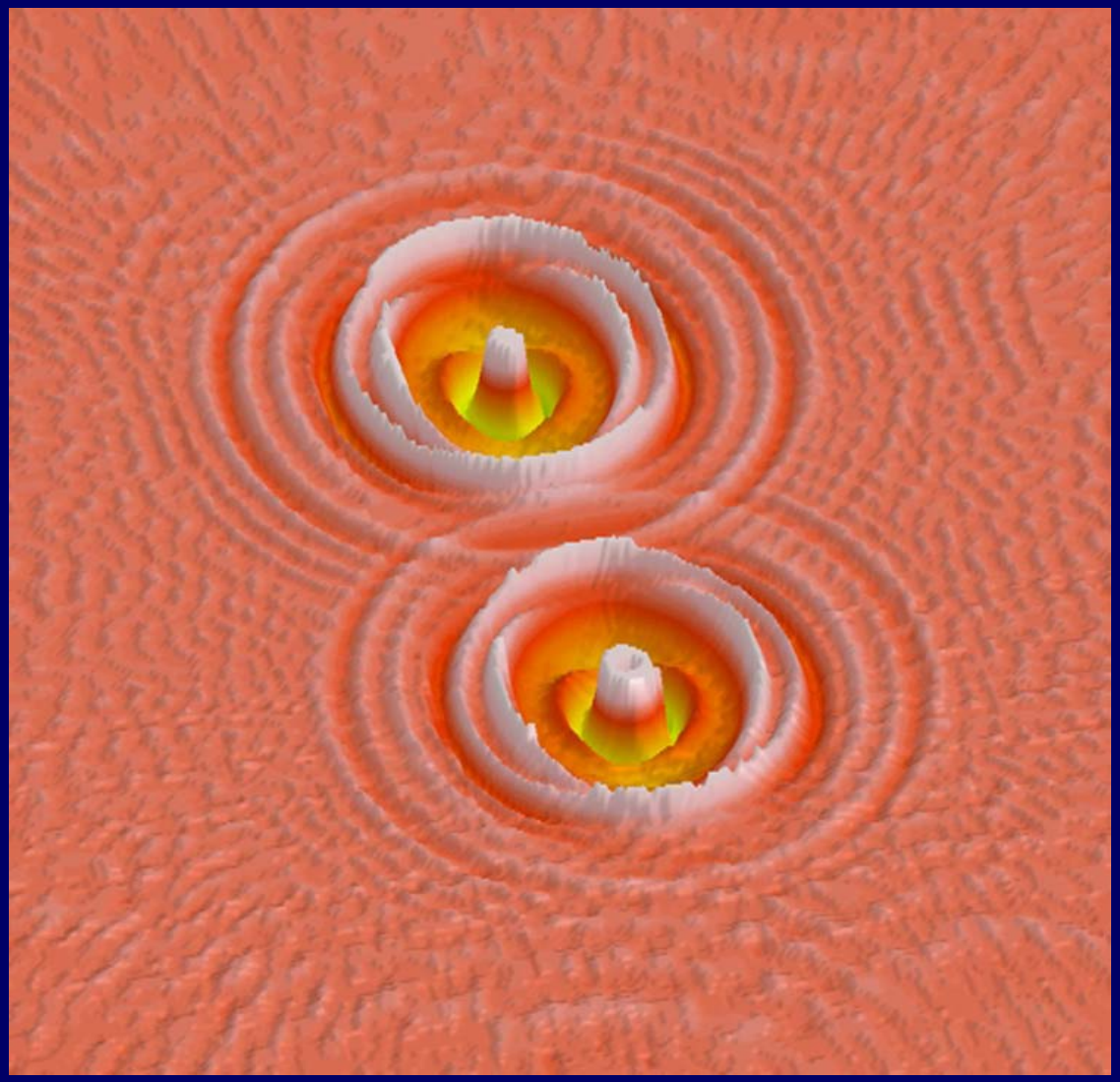
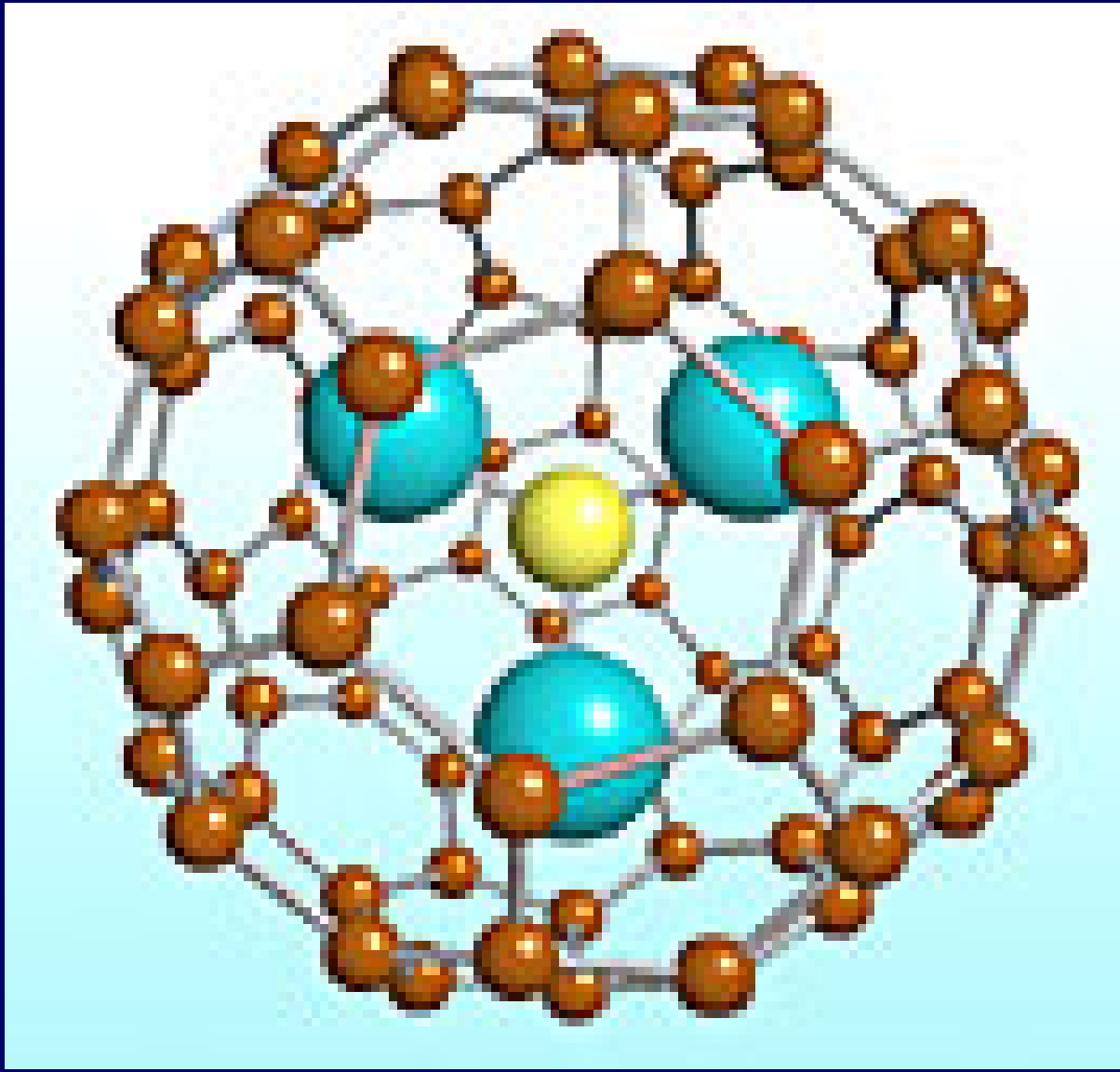


Credit: NIST

Special Medical Potential

- Carbon nanotubes and other particles
  - Packaging for pharmaceuticals and chemotheraputics (nanomedicines)
  - Reduction of systemic toxicity
  - Linkages to DNA or cancer cell components
- Quantum dots
  - Attached to specific cell receptors
  - Luminescence helps identify cancer cells
- Magnetic nanoparticles for imaging accuracy



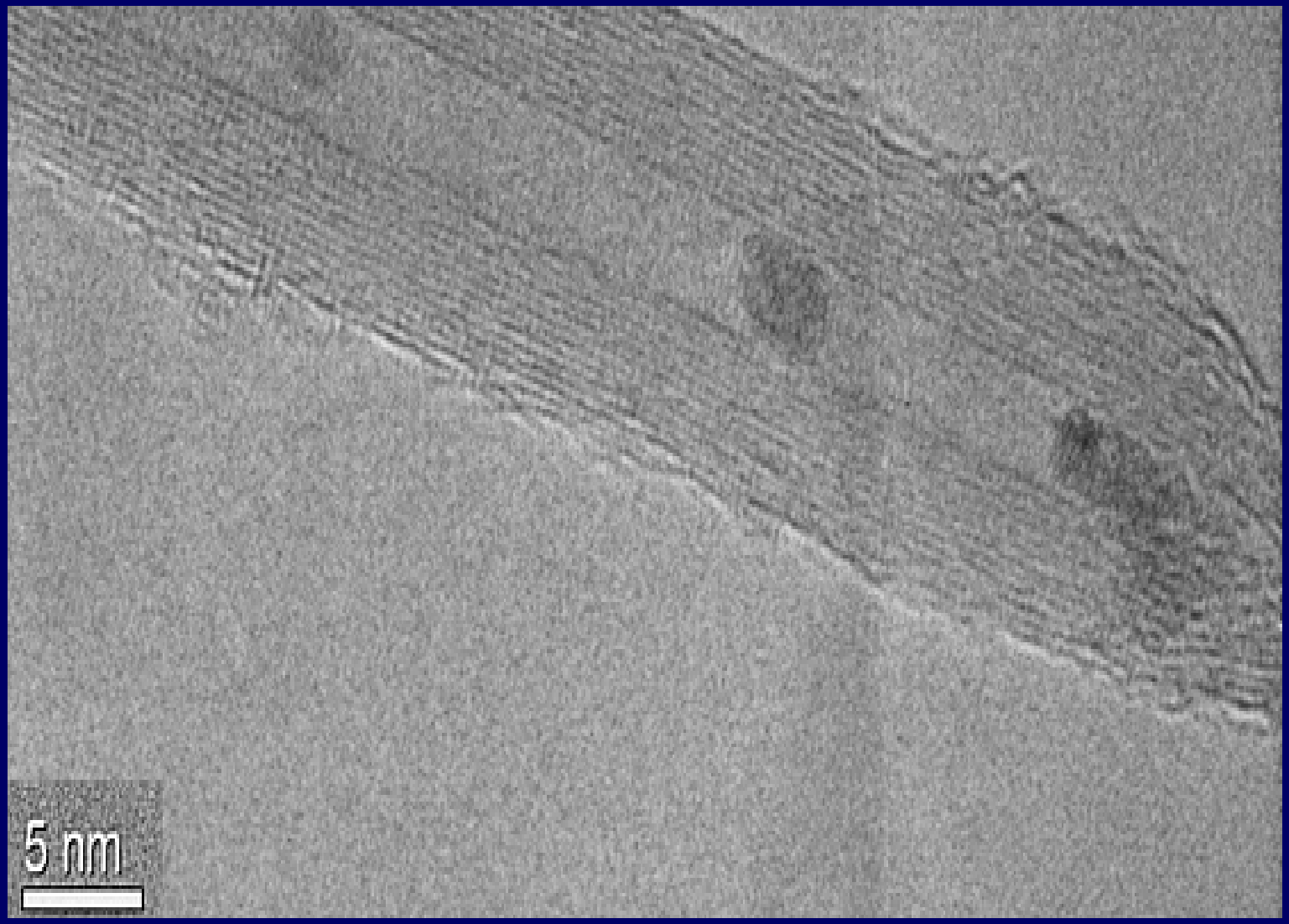


Image credit Nanolab Inc.

Market Prices

- “Hundreds of thousands per gram” initially
- 2005 - $100 per gram or $50,000 per pound
- 2008 - $30 to $1500 per gram
- Projected for 2010 to be <$20 per pound
- <$2 per pound when millions of tons produced annually

CHEAP TUBES, INC.

We search the world for the highest quality,
lowest cost carbon nanotubes,
so YOU don't have to!!!

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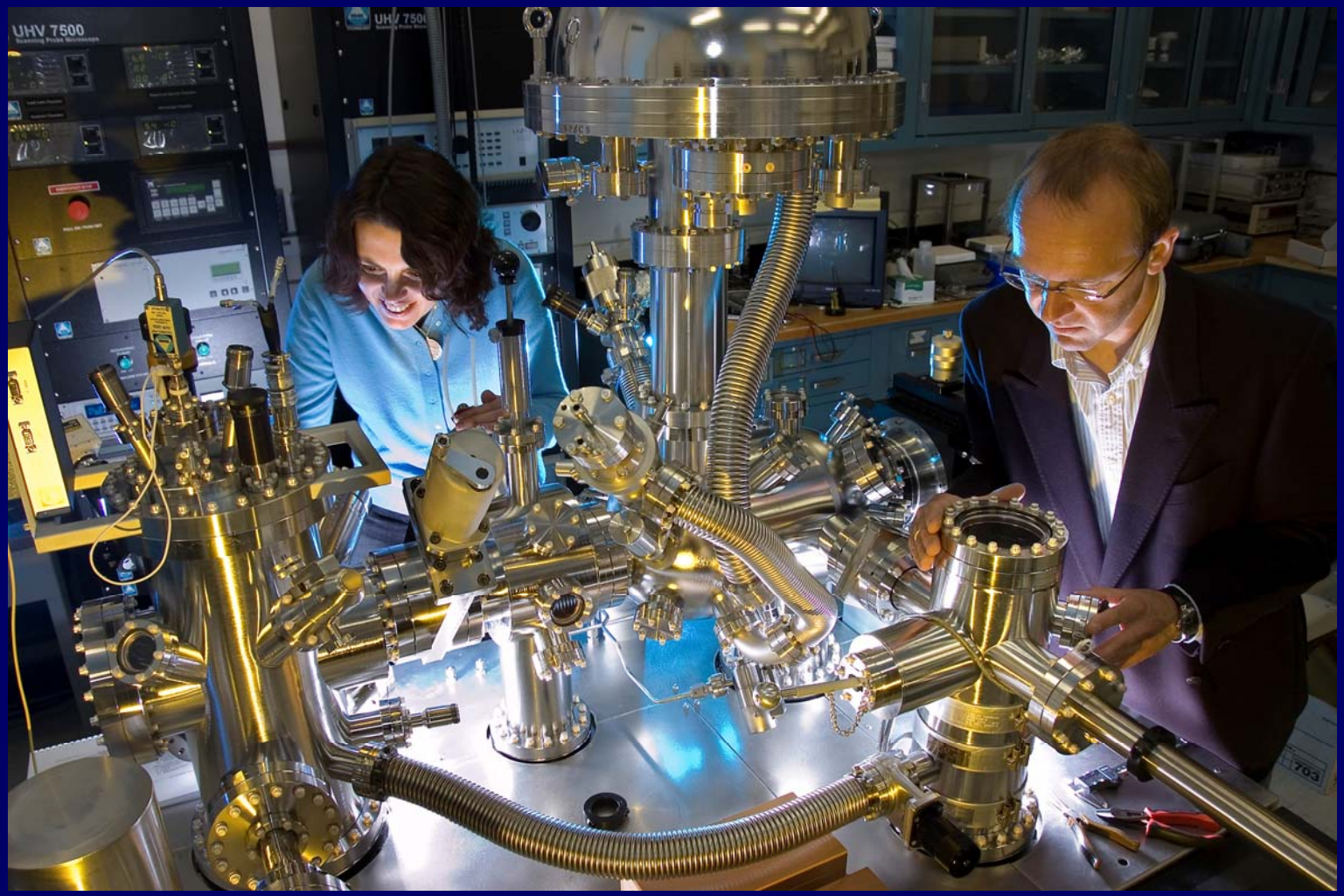
Nanoparticle exposure

- Not new
  - 10,000 year old Greenland ice – Neolithic Stone Age
  - 10^{12} (one trillion) submicron particles consumed per person per day
  - 5000 to 3 million particles/cm^3 in heavy traffic
  - Household gas appliances
- May be incidental (“natural”) or engineered
- Routes of exposure
  - Inhalation
  - Dermal
  - GI



Courtesy of Brookhaven National Laboratory

Inhalation Exposure

- Difficult to control background exposure for proper studies
- Nanoparticles naturally tend to aggregate
  - Smaller particles aggregate faster
  - Higher concentrations aggregate faster
- Aggregates deposit in upper airways
- Disaggergation may occur at cells or surfaces

Inhalation Exposure (cont.)

- Systemic translocation from the lung
  - To circulation via pulmonary vasculature
  - Lymph system
  - To GI tract from mucociliary apparatus
- Mixed study results:
  - Liver, spleen, heart, brain deposition of iridium particles in rats
  - No distribution of inhaled radio-labeled carbon nanoparticles in humans

Inhalation Exposure (cont.)

- Nasal sensory neurons
  - Animal evidence for olfactory nerve (and potentially CNS) accumulation
  - Difficult to interpret due to differences between humans and animals
- Known nasal sensory transportation of viruses
- High deposition of nanoparticles in human nasopharynx

Dermal Exposure

- Probably a significant route of exposure
- Sunscreens with TiO_2 nanoparticles
- MWCNT's can enter keratinocytes
- Little evidence for systemic absorption or penetration past the striatum corneum
- Damaged skin probably not a barrier

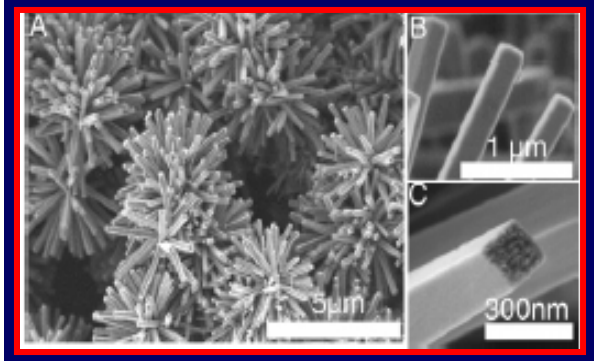
GI Exposure

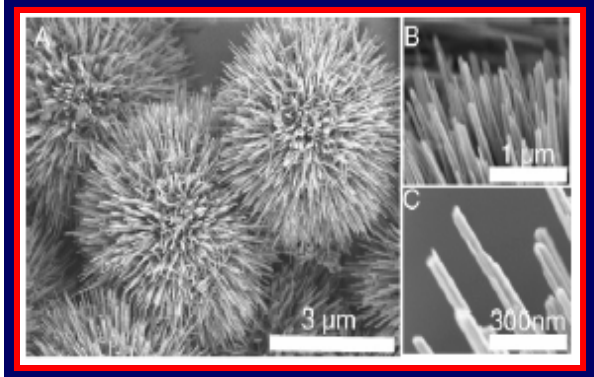
- May be a significant exposure pathway
  - Food, water
  - Swallowing inhaled particles
  - Hand-to-mouth transfer
- Absorption (and toxicity) governed by
  - Size
  - Surface characteristics
  - Water solubility

Other Exposures

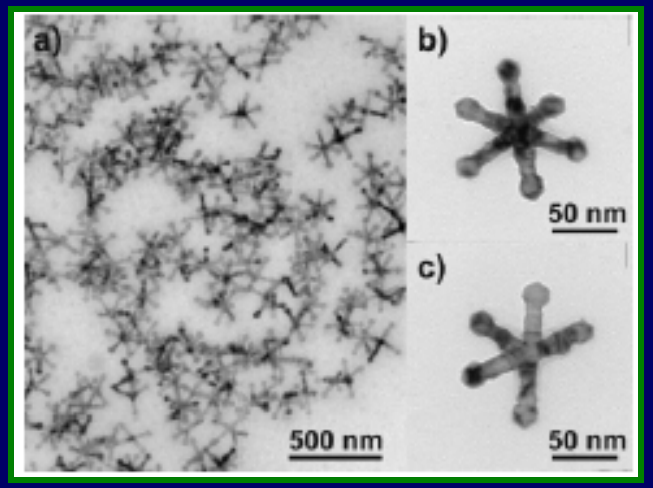
- Placental transfer of fullerenes
- Blood brain barrier
  - Fat soluble fullerenes
  - Olfactory nerve uptake

Literature Examples of MnO Jacks and Cubes

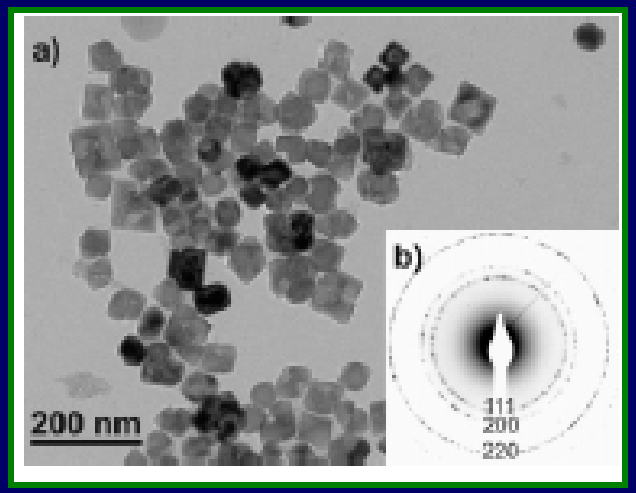


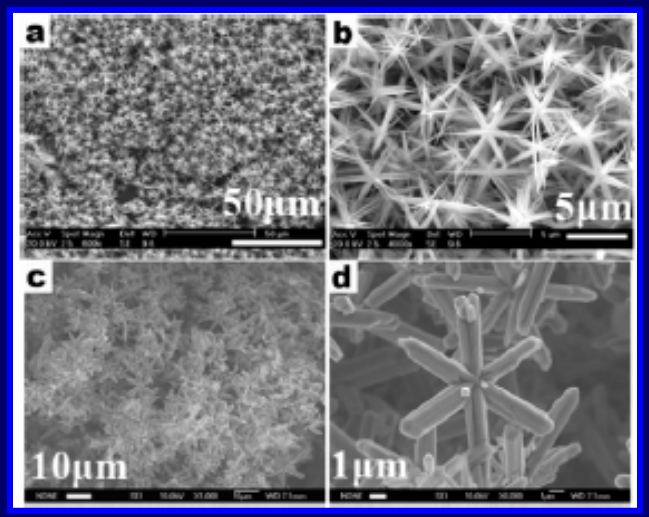


Jikang Yuan, et al. *JACS,* **2005**, 127, 14184.

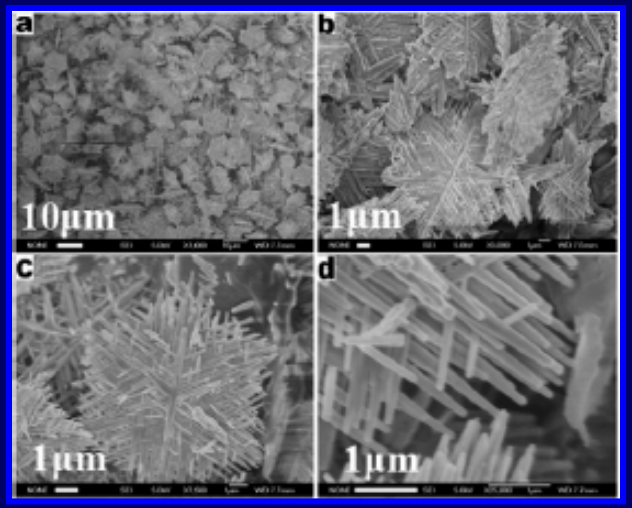


David Zitoun, et al. JACS, **2005**, 127, 15035.





Fangyi Cheng, et al. *Inorg. Chem.* **2006,** ASAP



See Paul Alivisatos, UCB

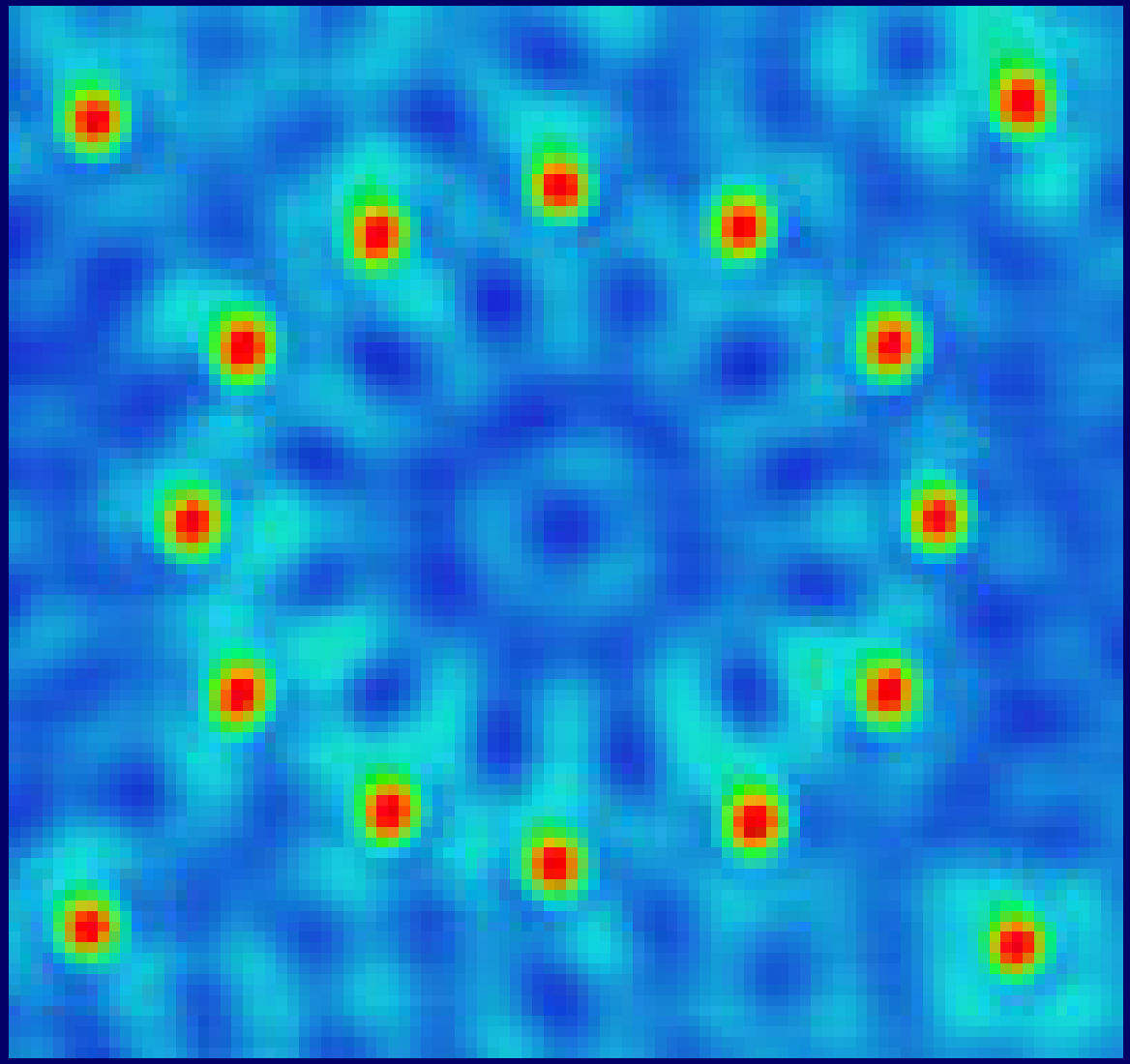
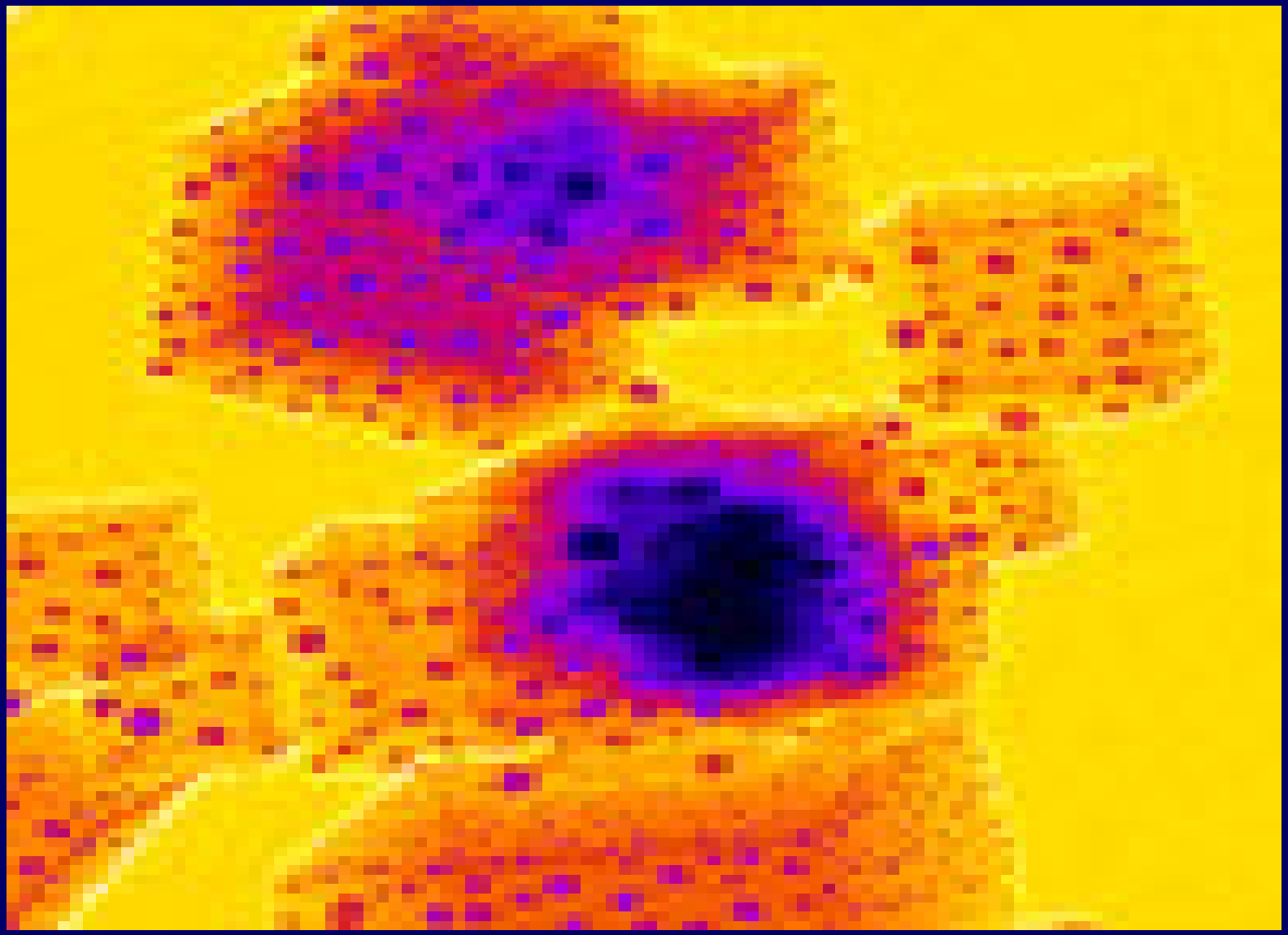


Image credit: Courtesy National Institute of Standards and Technology



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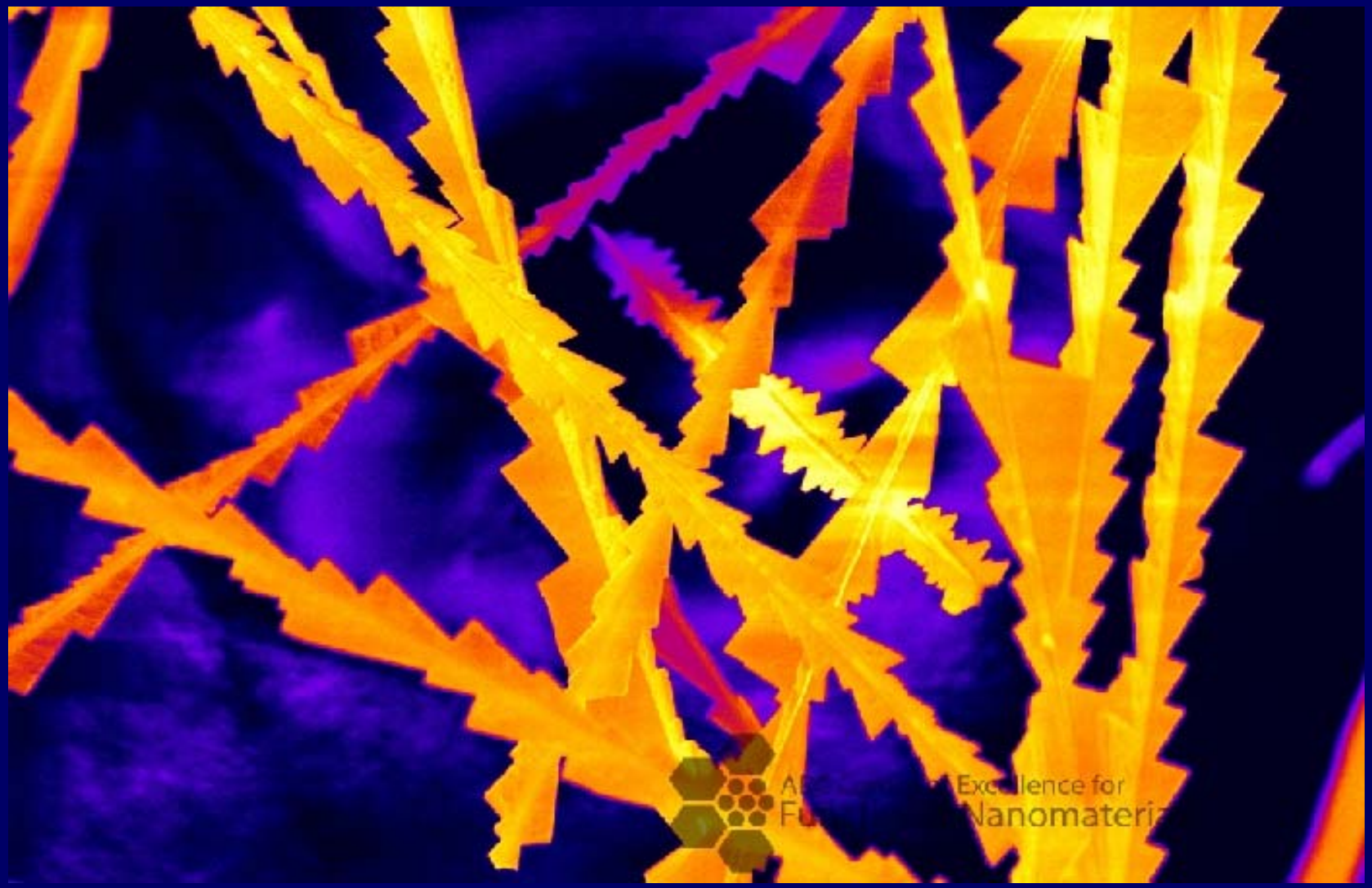


Image credit: ARC Center for Excellence in Functional Nanomaterials

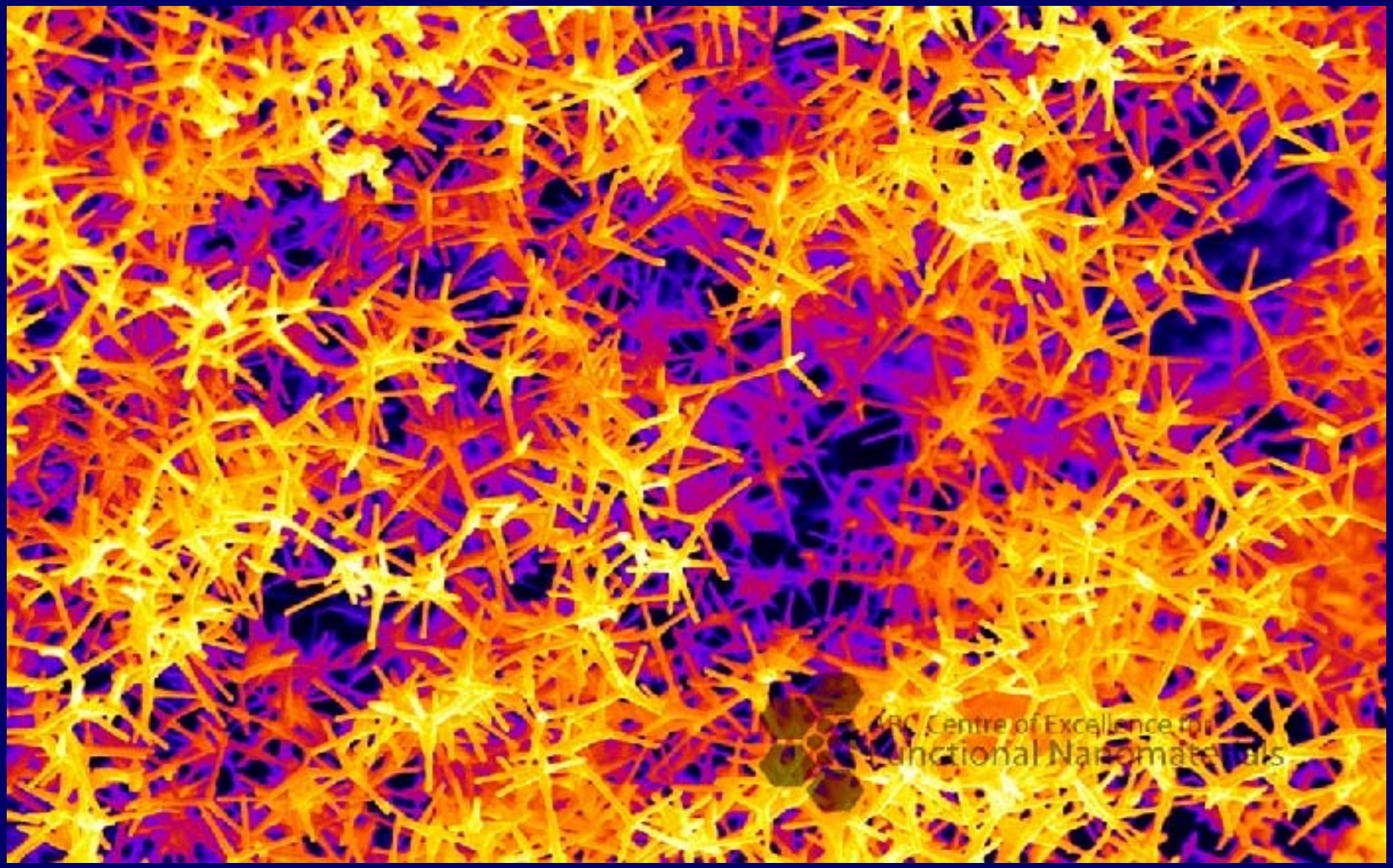


Image credit: ARC Center for Excellence in Functional Nanomaterials

Mechanisms of Toxicity

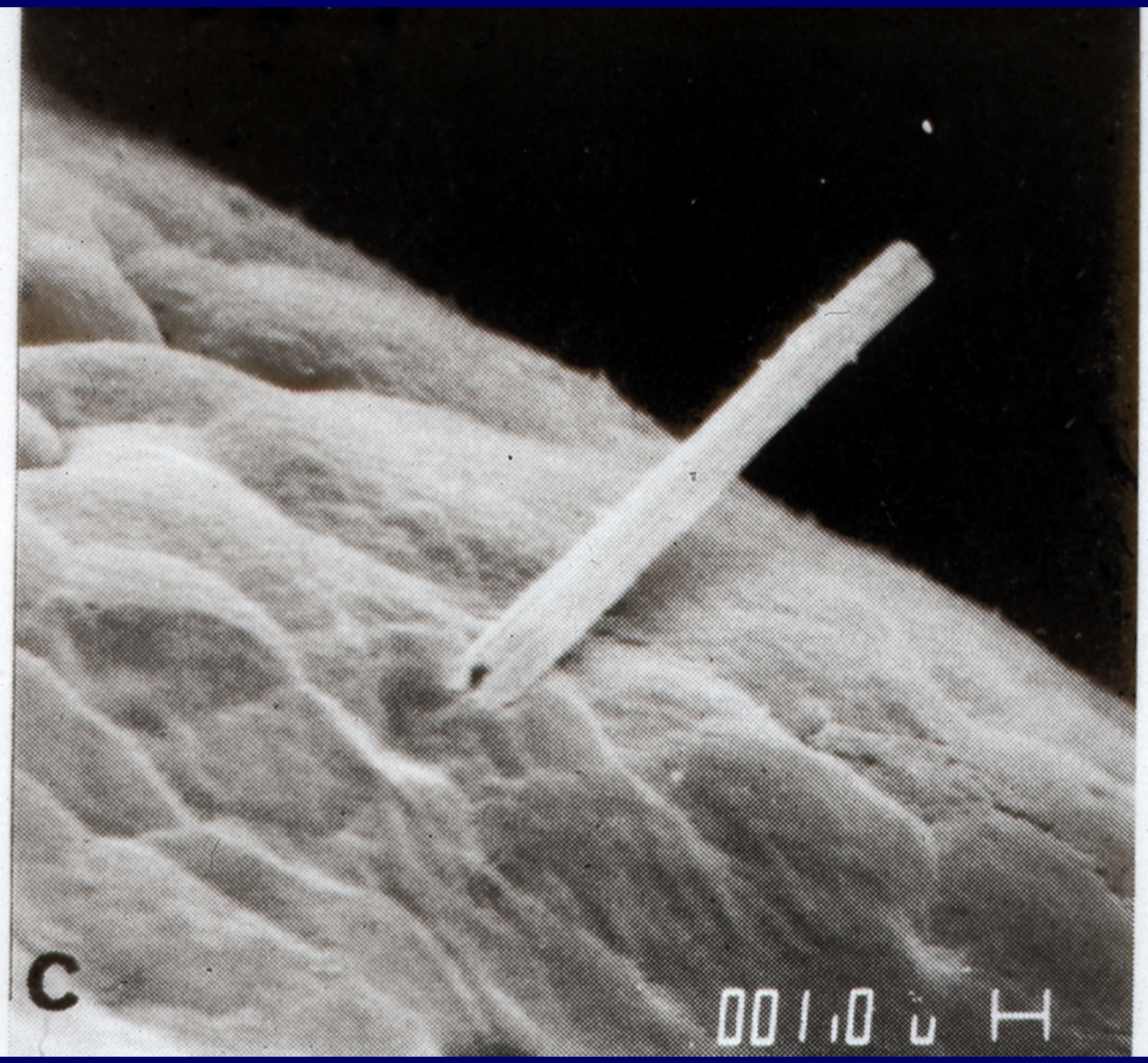
- Inflamation
- Cytotoxicity
- Antigenicity
- Genotoxicity

Nanotoxicities

- Pulmonary
- Cutaneous
- Systemic
- Carcinogenic

Pulmonary Toxicology

- Primarily inflammation
  - More dependent on surface characteristics
  - Aspect ratio
- Urban air pollution
  - Asthmatics
  - Cardiorespiratory mortality
  - Impaired respiratory function
- Biopersistence of MWCNT's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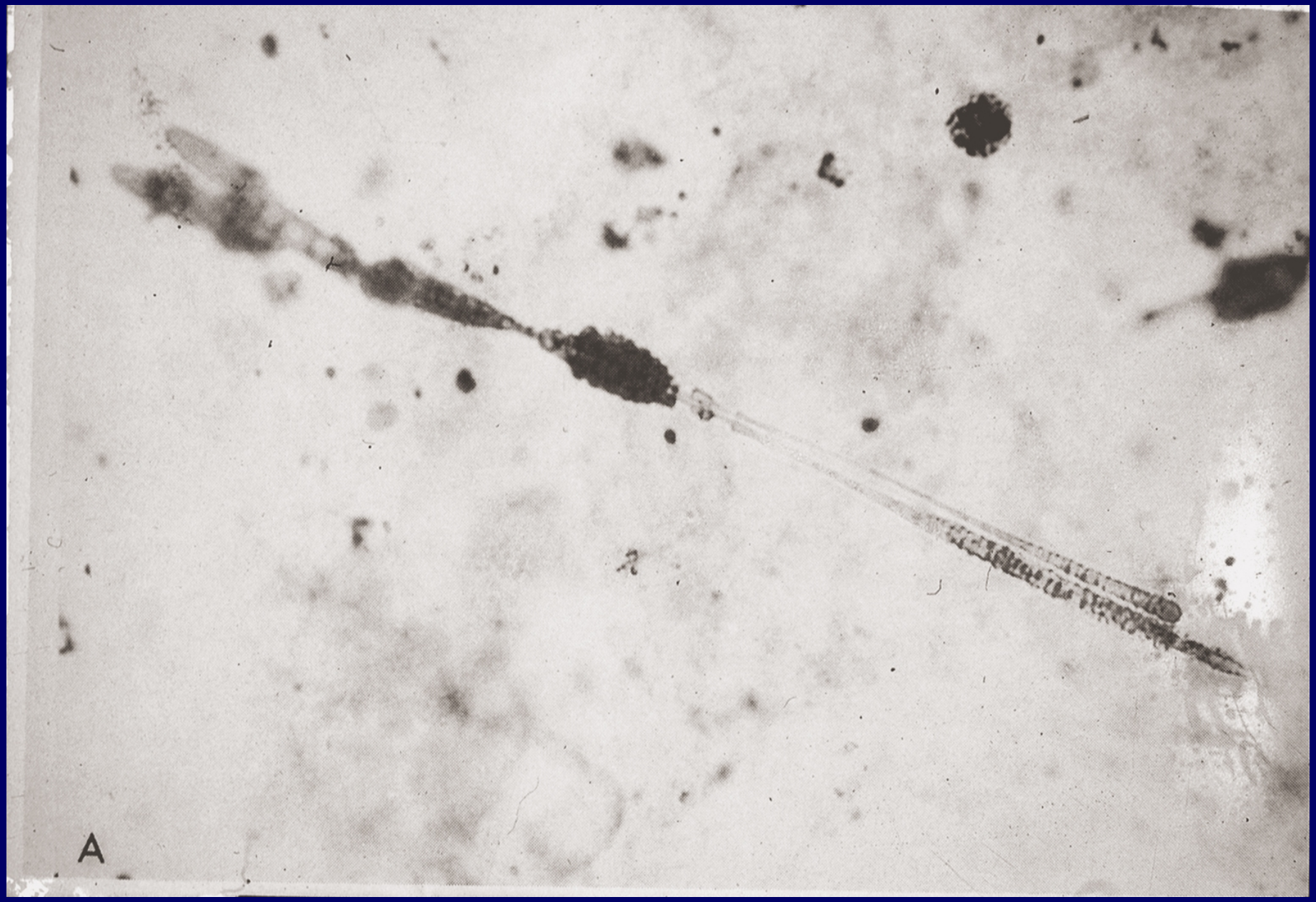
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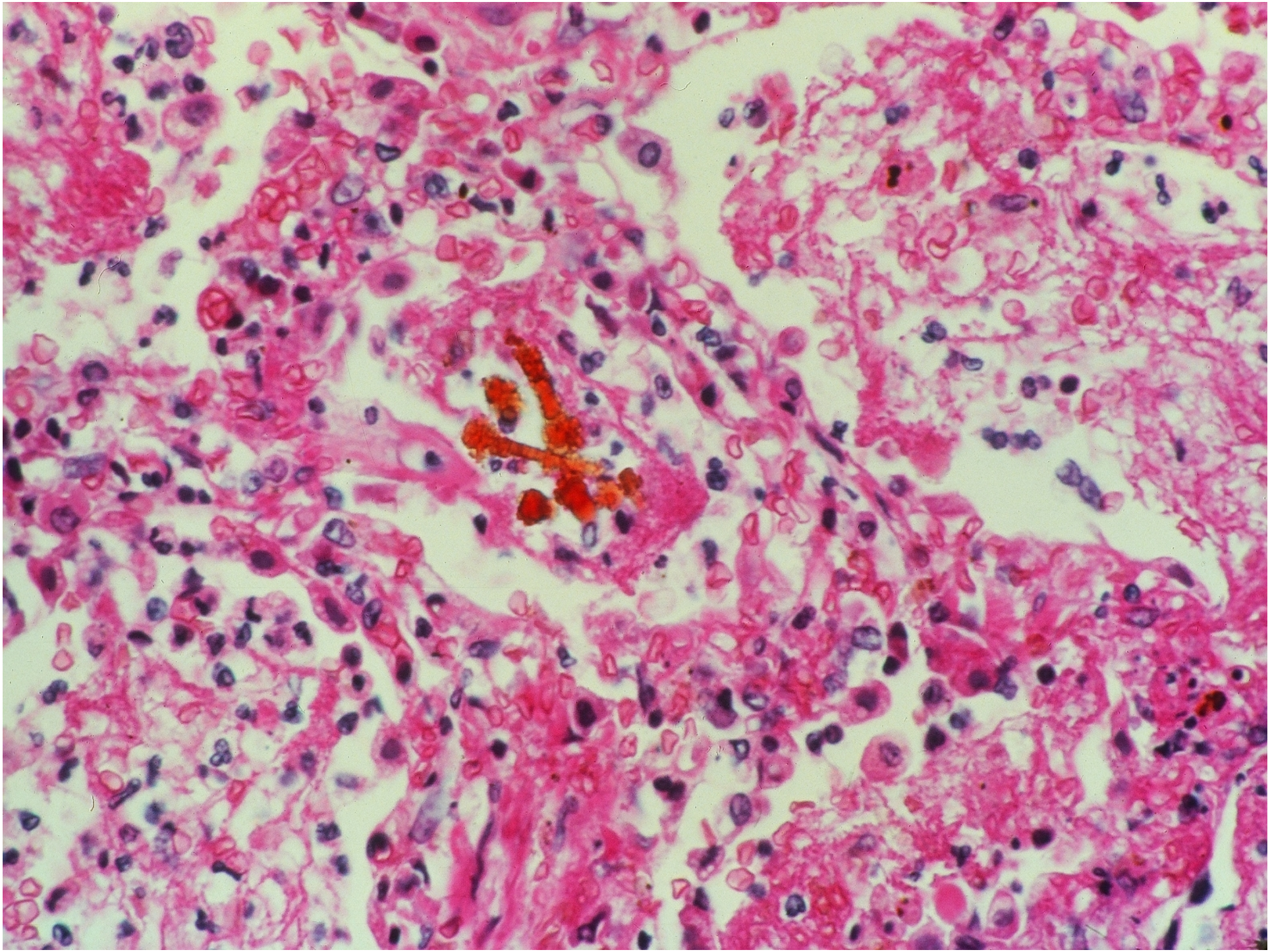
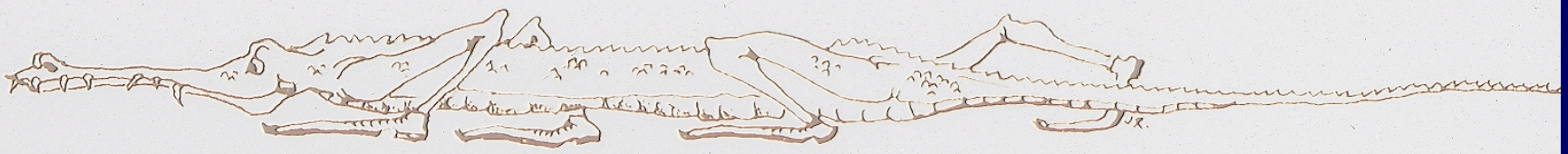


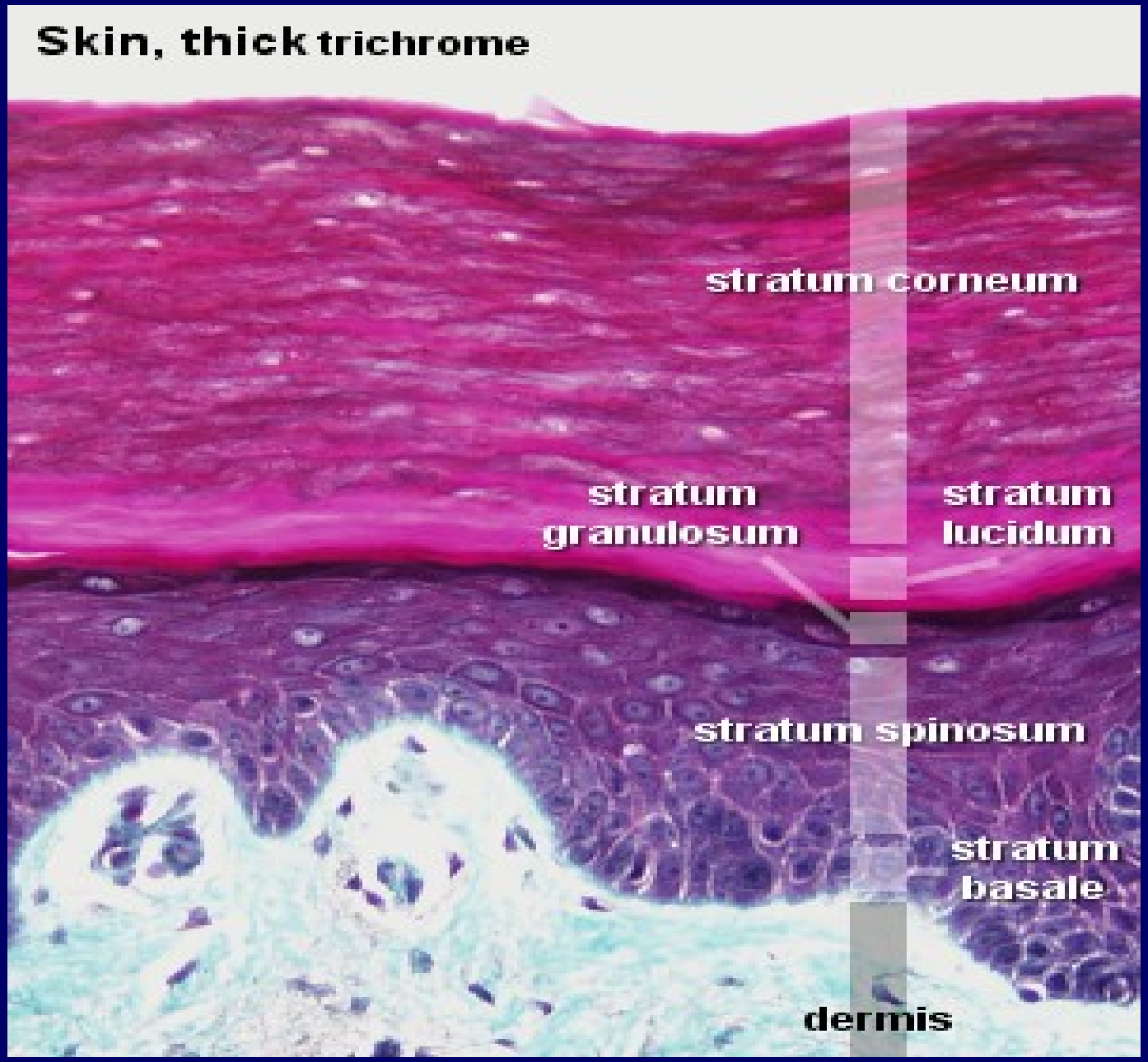


Pulmonary Toxicology (cont.)

- Extrapolating animals studies to humans
  - Intratracheal instillation
  - Obligatory nasal breathing of rodents
  - Lung overload
  - Contaminants
- Human epidemiology – TiO_2 workers
  - No increased lung cancer
  - No increased mortality

Cutaneous Toxicology

- Nanoscale metal oxides in sunscreen
  - Minimal irritancy
  - No sensitization
  - No photo-irritation
- Fullerenes, CNT's
  - No irritation
  - No patch test allergy
  - No ocular toxicity


Skin, thick trichrome
stratum corneum
stratum granulosum
stratum lucidum
stratum spinosum
stratum basale
dermis

Systemic Toxicology

- Limited acute studies
- Lack of chronic studies
- Acute studies – slightly to moderately toxic (based on oral LD50)
- Target organs
  - Lymphatics
  - Liver
  - Spleen
  - Kidney

Carcinogenicity

- Early studies just concluding
- Fullerenes
  - No dermal tumors in mice
  - Negative in Ames assay (non-mutagenic)
  - Anti-mutagenic in chromosome aberration tests
  - Photo-activation mutagenicity
  - Photo-activation cytotoxicity

Environmental Hazards

- Concern about rapid distribution and bio-accumulation
- Dispersion limited due to rapid surface absorption and low water solubility
- Requires individual nanomaterial assessments due to widely variable characteristics

Characteristics that Modify Hazard

- Size
- Surface area
- Solubility
- Surface characteristics and reactivity
- Shape and aspect ratio
- Agglomeration states
- Contamination

Risk Assessment

- Hazard identification
  - Potential pulmonary and systemic toxicity
- Dose response assessment
  - Intratracheal injection not like inhalation
  - Chronic inhalation studies needed
- Exposure risk assessment
  - Respirable particles
- Risk characterization
  - Toxic dose

Regulatory Issues

- Current regulatory status
  - EPA, OSHA, NIOSH, NTP & FDA research agendas
  - Are nanomaterials “new chemicals” triggering independent EPA review?
- Current OSHA PEL’s for nanoparticles
  - Asbestos
  - Carbon black
  - Metal fumes and dusts
  - “nuisance dust”
  - Graphite (where PEL’s currently fall)
- Personal Protective Equipment (PPE)

Material Safety Data Sheet for Carbon Nanotubes (CNTs)

Component	%	OSHA/PEL	ACGIH/TLV
Synthetic graphite	Up to 100%	15 mg/m3 (total dust) 5 mg/m3 (respirable fraction)	2 mg/m3 TWA
Metallic impurity	Balance		

Potential Health Effects

Eye Contact: May cause eye irritation
Skin Contact: No known hazards, but may be mildly irritating
Inhalation: May cause irritation to respiratory tract
Ingestion: No known hazards, but may irritate gastrointestinal tract
Acute and Chronic High concentration of dusts may be irritating to eyes, skin,
Health Effects: mucus membranes and respiratory tract.

Regulatory Issues – recent decisions

- EPA
  - nanomaterials not “new” compounds
- British Soils Association
  - Ban on engineered nanoparticles <200 nm in organic food

Future Issues

- Nanomaterials are hugely diverse
- Need to accurately characterize nanoparticles
- Need physiologic and chronic exposure studies
- Increased biocompatibility may increase exposure and environmental persistence