SWNT Interactions with Biological Systems: Focus on Length

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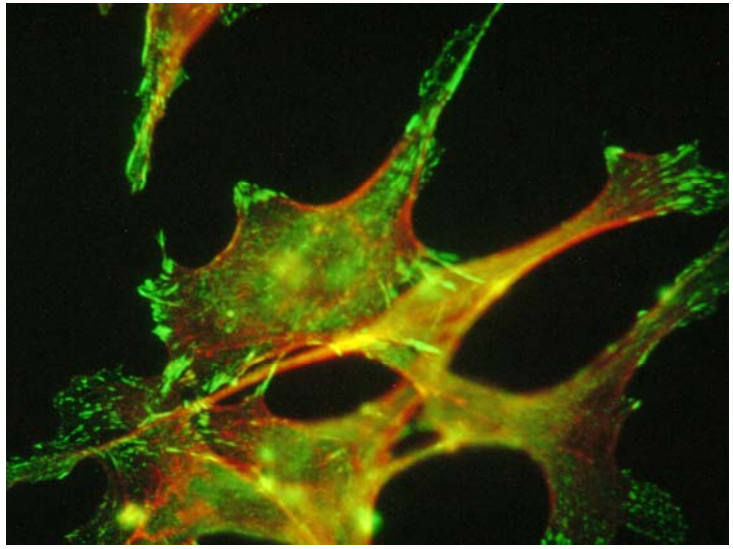

Why Now…Potential for Catastrophic Effects: Psychological and Economic

Flawe

Posted May 28, 20

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about the good r

NANOTECHNOLOGY

EPA Ponders Voluntary Nanotechnology Regulations

Last week, the U.S. Environmental Protection Agency (EPA) held its first public meeting to gauge sentiments about a proposed voluntary pilot program to collect information on new nanomaterials that companies are making. The agency got an earful.

More than 200 people gathered here at the Washington Plaza hotel to weigh in on the program, a possible precursor to guidelines that would mark the agency's first attempt to regulate nanotechnology. In a document released before the meeting, a coalition of 18 environmental and health-advocacy groups charged that a voluntary program would be inadequate to protect people from new chemical hazards. But most makers of nanomaterials applauded EPA's initial move as appropriate, because so little is known about the possible hazards of nanosized particles.

"The meeting was like the blind man feeling the elephant," says David Rejeski. He heads a new 2-year project at the Woodrow Wilson International Center for Scholars in Washington, D.C., on managing health and environmental impacts of nanotechnology. EPA and other agencies are still sorting out the scale of the challenge they face, Rejeski says.

Nanomaterials put regulators in an unfamiliar bind. With traditional chemical toxins, any two molecules with the same chemical formula look and behave alike. Two nanoparticles made of the same elements but of different sizes, however, may have drastically different chemical properties. Even particles of the same size and elemental composition can have very different properties, due to differences in their chemical architecture—for example, diamond nanocrystals and buckyballs shaped like soccer balls, both made of pure carbon. That diversity makes it a daunting task to sort

Hazardous? The sheer diversity of nanoparticles makes it hard to tell which ones pose risks.

As a first step, EPA is thinking about asking nanomaterials makers to submit information on just what they are producing, how much is made, and possible worker exposure. "That's a good first step," says Sean Murdoch, executive director of the NanoBusiness Alliance in Chicago, Illinois. But Jennifer Sass of the Natural Resources Defense Council in Washington, D.C., argues that asking companies to participate voluntarily doesn't go far enough. "It's going to be tough getting these companies to be good corporate citizens without the threat of regulation hanging over their heads," Sass notes.

Nearly everyone agrees that far more information is needed. To get it, some groups are starting to call for increased funding for toxicity and health studies on nanoparticles. In a commentary in the 14 June *Wall Street Journal*, Fred Krupp, president of Environmental Defense, and Chad Holliday, chair and CEO of DuPont, argued that funding for environmental health and safety studies of nanotechnology should rise from its current level of 4% to 10% of the $1.2 billion budget of the National Nanotechnology Initiative.

currently drawing up just such an analysis and plans to release it later this summer.

–ROBERT F. SERVICE

With reporting by Amitabh Avasthi.

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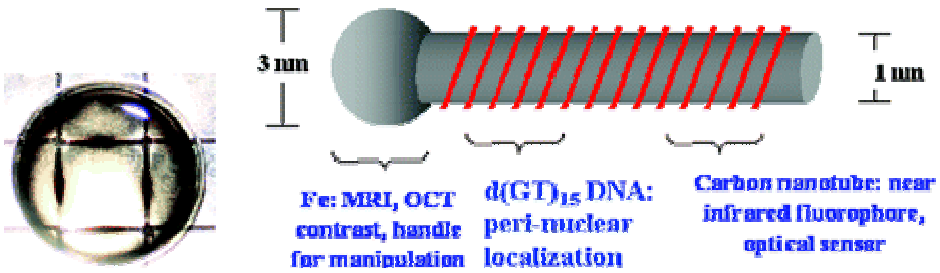
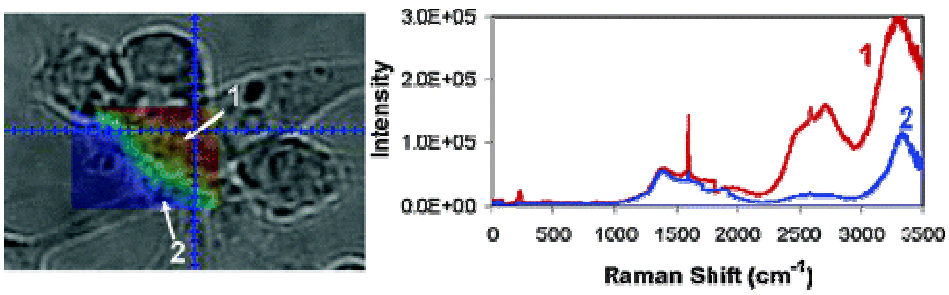
This approach will not reassure the public at large

Published by AAAS

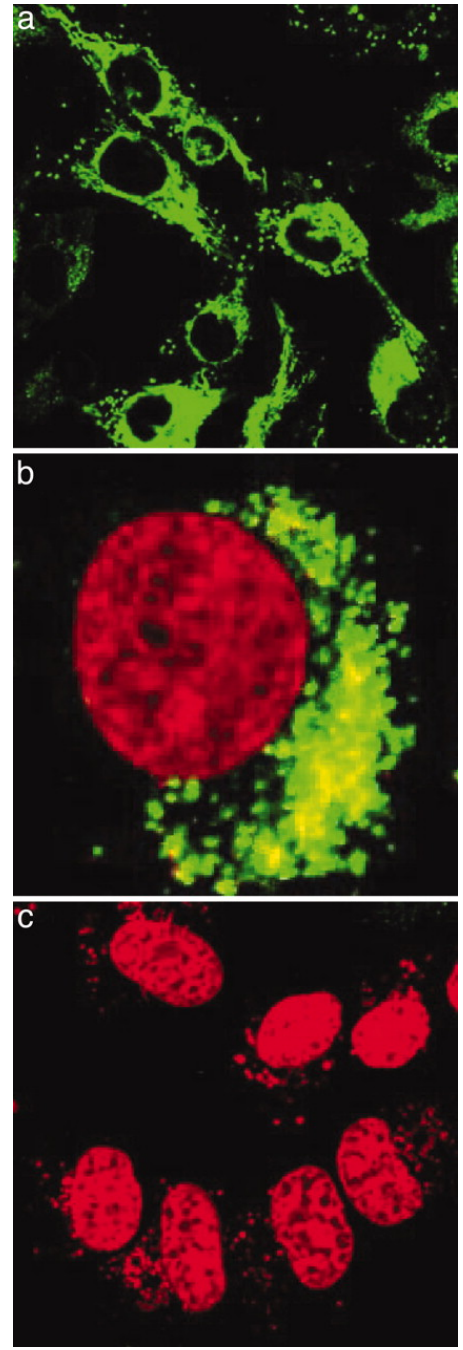
Why are we interested in bio…

Potential for great economic benefit





(a) (b)



Labeled DNA SWNTs

Labeled Nuclei & DNA SWNTs

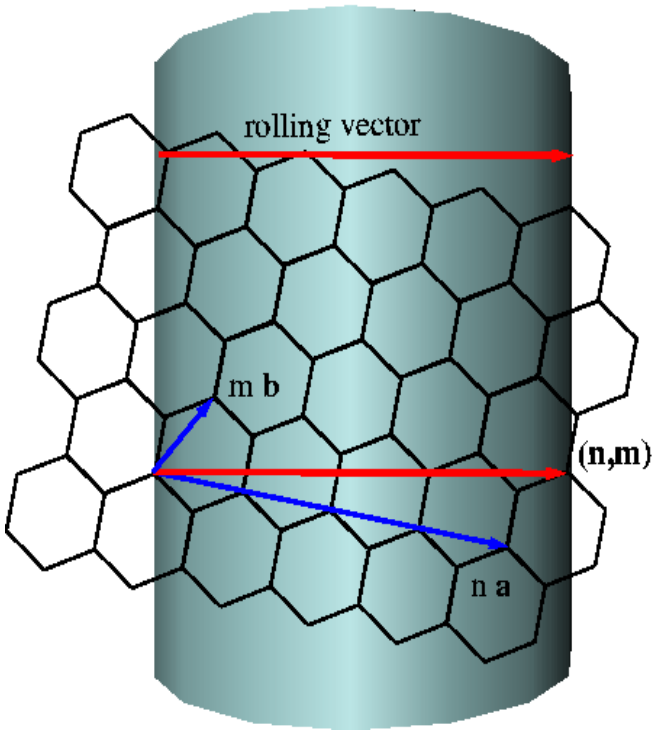
Nuclei

MS Strano et al. Nano Letters, **2007**, *7(4),* 861 -867.

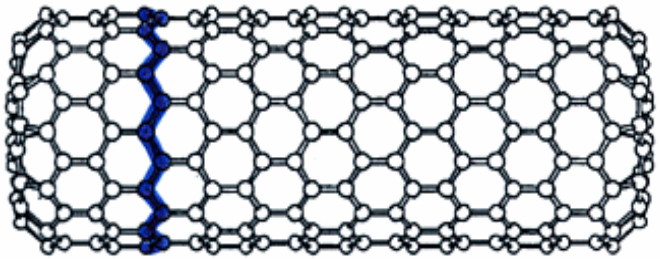
Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction
Dai et al, PNAS, **2005**, *102(33),* 11600-11605.

SWNTs Present Many Characterization Challenges

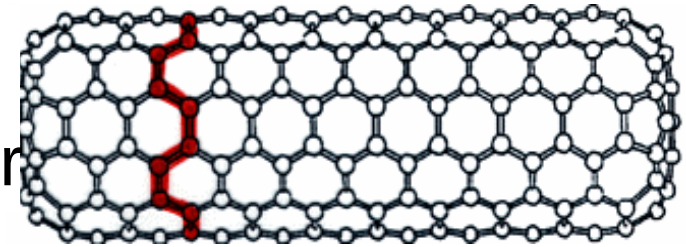
Carbon nanotubes are basically sheets of graphite rolled up into tubes with diameter range (0.4 – 4) nm.



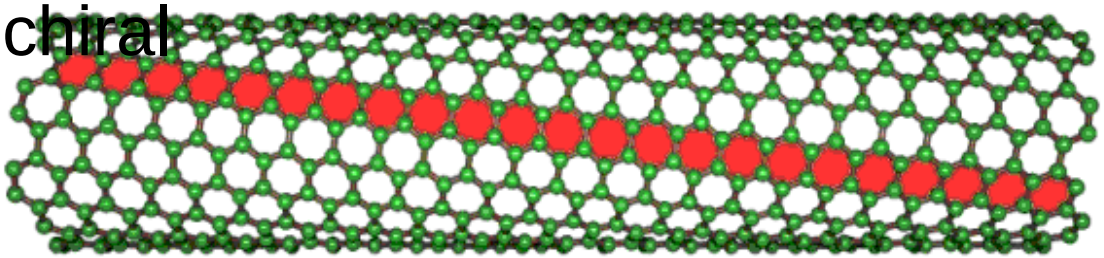
(n,0)
zigzag



(n,n)
armchair



(n,m)
chiral



What makes SWNTs so difficult to work with ?

1. Innate material heterogeneity
2. Poor solubility – in anything
3. Large "inter-particle" interactions
4. Few chemical handles – without creating new ones

What is required for Biological use..

1. Extensive separation efforts
2. Wrapping "polymer" required for solubility
3. Derivatize with functional species if needed
4. Characterize structural, spectral & solution properties

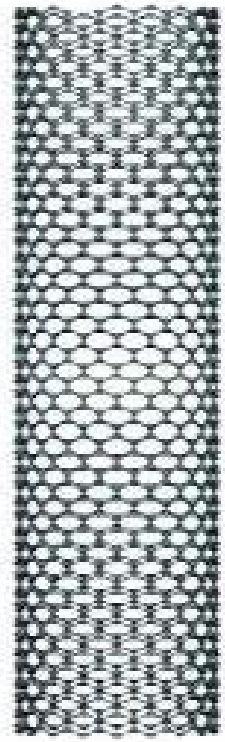
Structural & Physical Properties of Nanomaterials in General

Tube length

surfactant thickness

Molecular mass
& distribution

Chirality



Tube width

Defects

Surface chemistry

Extent of bio-functionality

Properties Impacted by Biologically Relevant Measurable Parameters

Solubility
Dispersion
pH susceptibility
Bioactivity
Diffusion coefficient
Anisotropic transport
Membrane permeability
Degradation mechanisms and byproducts

The Problem: Conflicting Reports in the Literature

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Oops They Did It Again! Carbon Nanotubes Hoax Scientists in Viability Assays

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ABSTRACT

New materials of emerging technological importance are single-walled carbon nanotubes (SWCNTs). Because SWCNTs will be used in commercial products in huge amounts, their effects on human health and the environment have been addressed in several studies. Inhalation studies in vivo and submerse applications in vitro have been described with diverging results. Why some indicate a strong cytotoxicity and some do not is what we report on here. Data from A549 cells incubated with carbon nanotubes fake a strong cytotoxic effect within the MTT assay after 24 h that reaches roughly 50%, whereas the same treatment with SWCNTs, but detection with WST-1, reveals no cytotoxicity. LDH, FACS-assisted mitochondrial membrane potential determination, and Annexin-V/PI staining also reveal no cytotocicity. SWCNTs appear to interact with some tetrazolium salts such as MTT but not with others (such as WST-1, INT, XTT). This interference does not seem to affect the enzymatic reaction but lies rather in the insoluble nature of MTT–formazan. Our findings strongly suggest verifying cytotoxicity data with at least two or more independent test systems for this new class of materials (nanomaterials). Moreover, we intensely recommend standardizing nanotoxicological assays with regard to the material used: there is a clear need for reference materials. MTT–formazan crystals formed in the MTT reaction are lumped with nanotubes and offer a potential mechanism to guide bioremediation and clearance for SWCNTs from "contaminated" tissue. SWCNTs are good supporting materials for tissue growth, as attachment of focal adhesions and connections to the cytoskeleton suggest.

Introduction. Nanoparticles and nanomaterials can be on the same scale as elements of living cells, including proteins, nucleic acids, lipids and cellular organelles. When considering nanoparticles it must be asked how man-made nanostructures can interact with or influence biological systems. On one hand, nanosystems are specifically engineered to interact with biological systems for particular medical or biological applications. On the other hand, the large-scale production of nanoparticles for either nonmedical applications or as the side products of combustion processes may affect a wide range of organisms throughout the environment. There have been an increasing number of investigations concerning the use of nanoscale structures since the 1970s; for example, liposomes for drug transport and comparable applications have been undertaken.[1−5] In addition to liposomes, nanoparticles produced from other materials came into the focus of physicians for various disease treatments.[6,7] The goal of these works has been to design inert auxiliary accompanying materials and to use body-friendly and biodegradable excipients. However, dependent on their target organ and functionality, not all of these materials are degradable and some stay in the body for long periods of time. Thus, long-term side effects and foreign body reactions may be detectable and a good local and systemic tolerance during and after medication should be a condition *sine qua non*. Nanostructured materials come into contact with biological systems through their use in drug delivery systems or for gene transfer. They are also produced for food and cosmetic chemistry and many other technical applications.[8] The increasing production, particularly of single-walled carbon nanotubes, will enhance the possible exposure at workplaces, packing stations, and during application of these products.[9] In addition, waste treatment and containment at the end of a product's life cycle must be considered. Because of all of these reasons, it is of great interest to determine how these materials, when coming into contact with living organisms, are taken up, transported in or through cell layers,[10] and possibly affect biological functions. In this report we focus on the toxicity of single-walled carbon nanotubes (SWCNTs) with their unique functions for electronics, engineering and biomedical applications, and as sensors. Among the new carbon modifications described

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10.1021/nl060177c CCC: $33.50 © 2006 American Chemical Society
Published on Web 04/27/2006

Many reports have real flaws

Highly heterogeneous

Undefined dispersion

Poor surface characterization

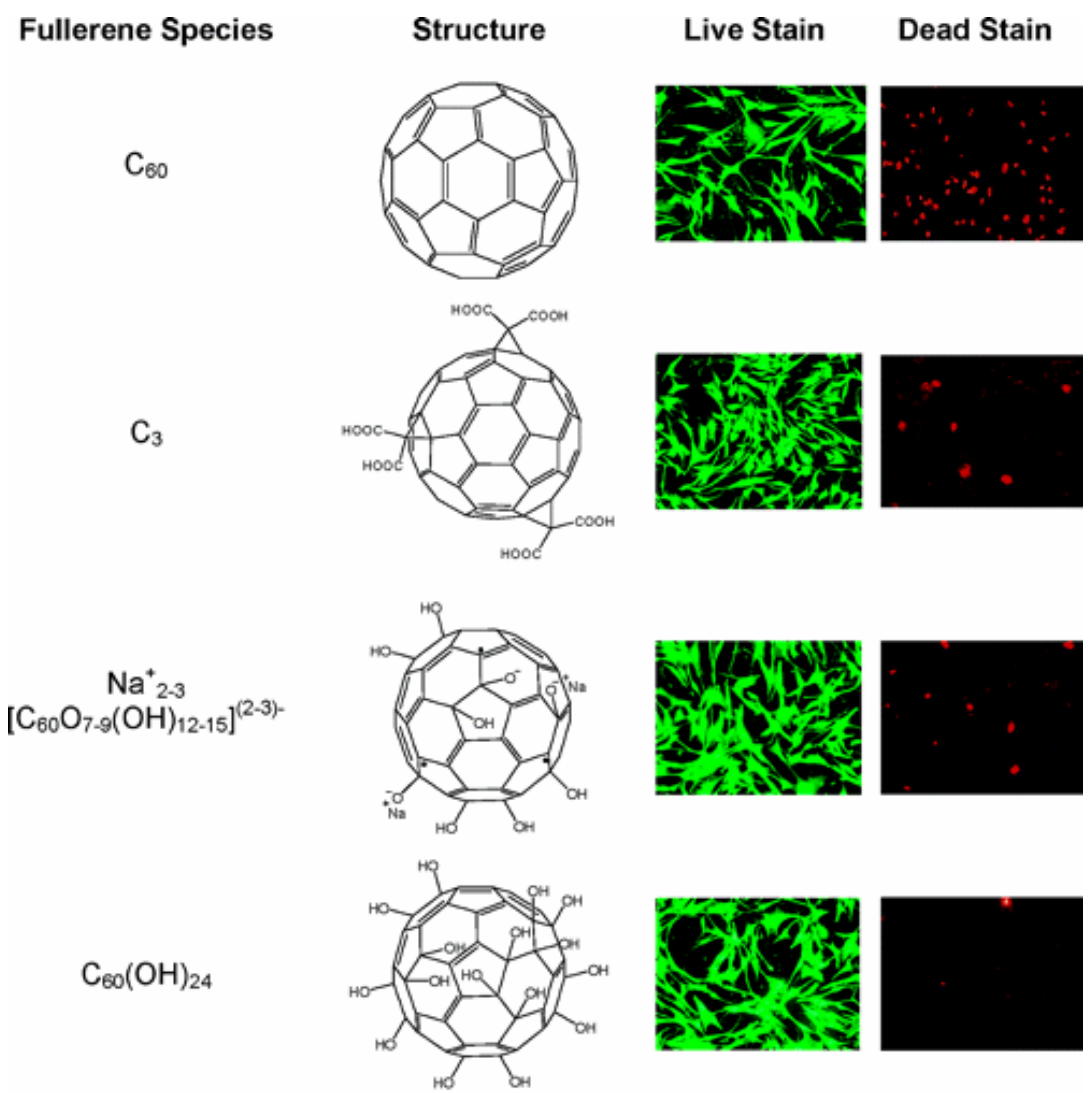
Undefined surface charge

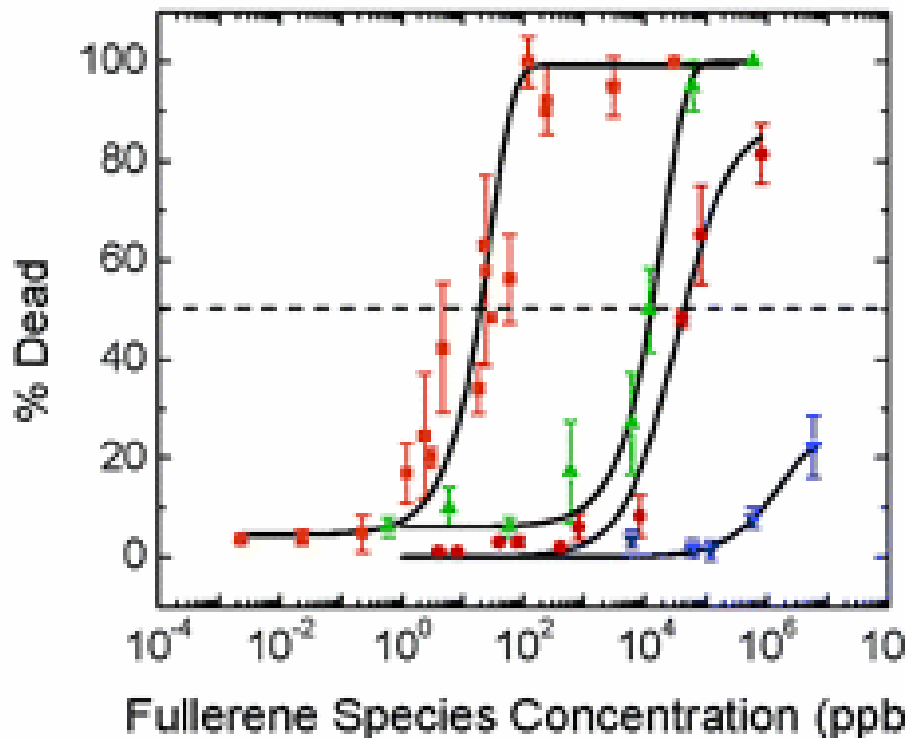
No length characterization

Sweeping conclusions

Heavily reliance on "kits"

A high level of precision is required for a meaningful bio-measurement





Variable surface chemistry induces

6 orders of magnitude

difference in toxicity profile

CM Sayes, JD Fortner, W Guo, D Lyon, AM Boyd, KD Ausman, YJ Tao, B Sitharaman, LJ Wilson, JB Hughes, JL West, & VL Colvin
"The Differential Cytotoxicity of Water-Soluble Fullerenes" *Nano Letters,* 4 (10), 1881 -1887, 2004

Preparative Method Has Distinct Effects on Resulting Properties

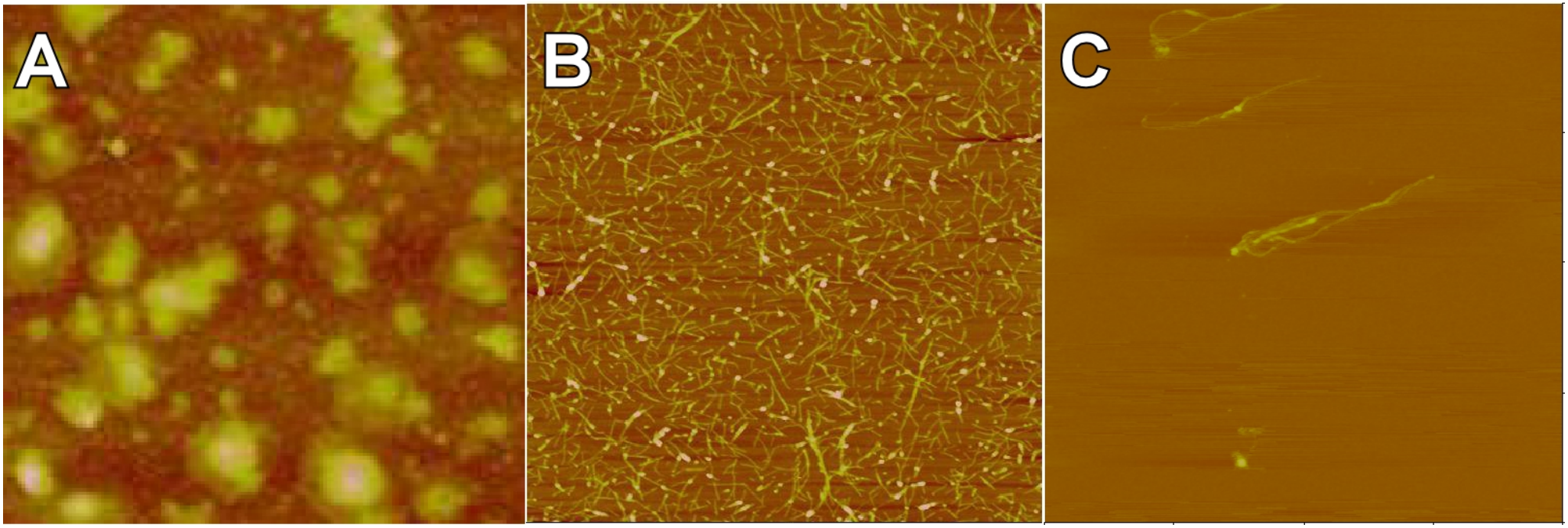
Prep Methods

Dispersion

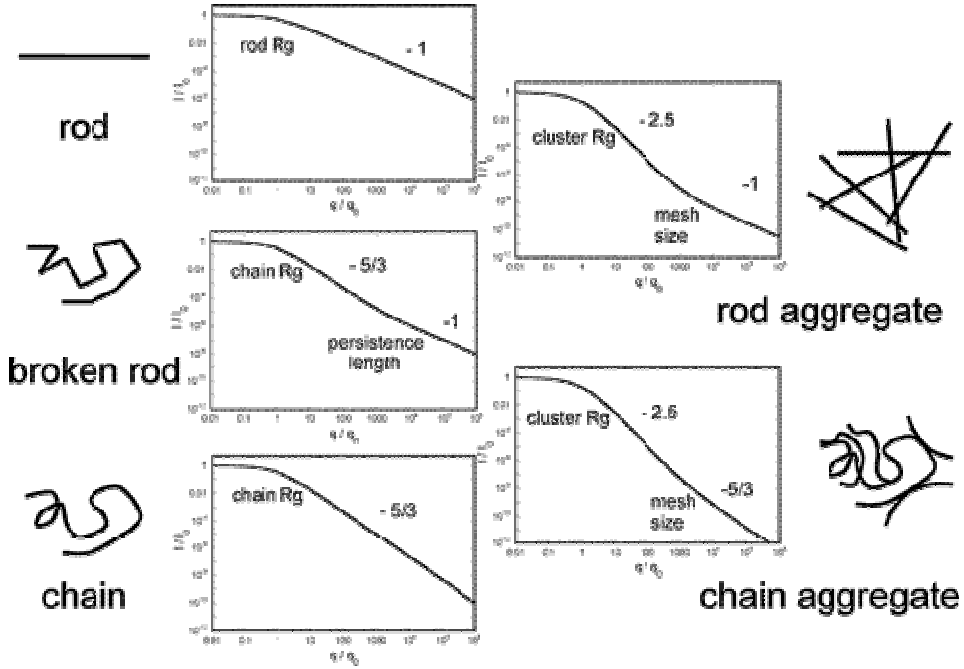
Metal Content

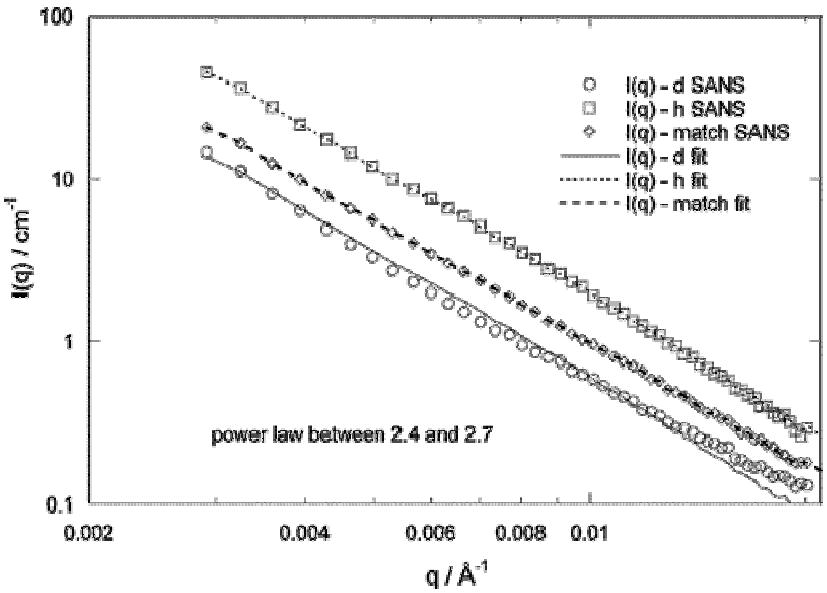
Uptake

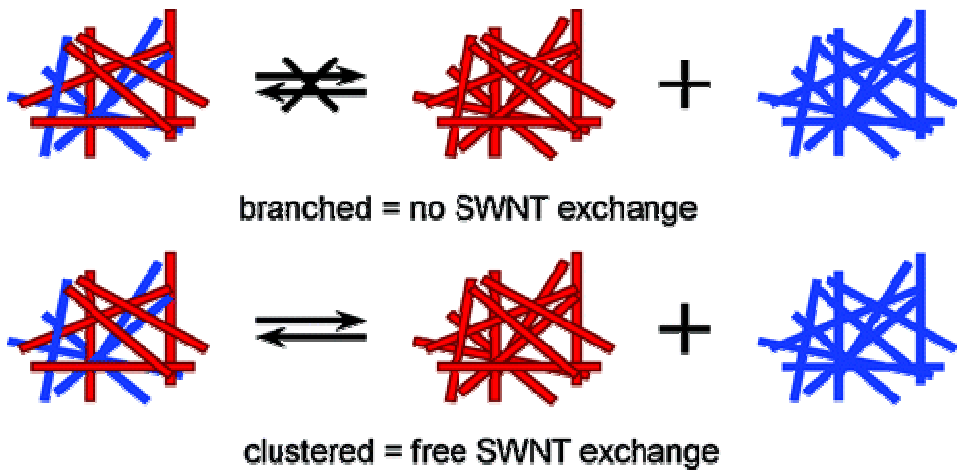
(A) SWNT-ODA
(B) SWNT/DNA
(C) SWNT-butyl graft

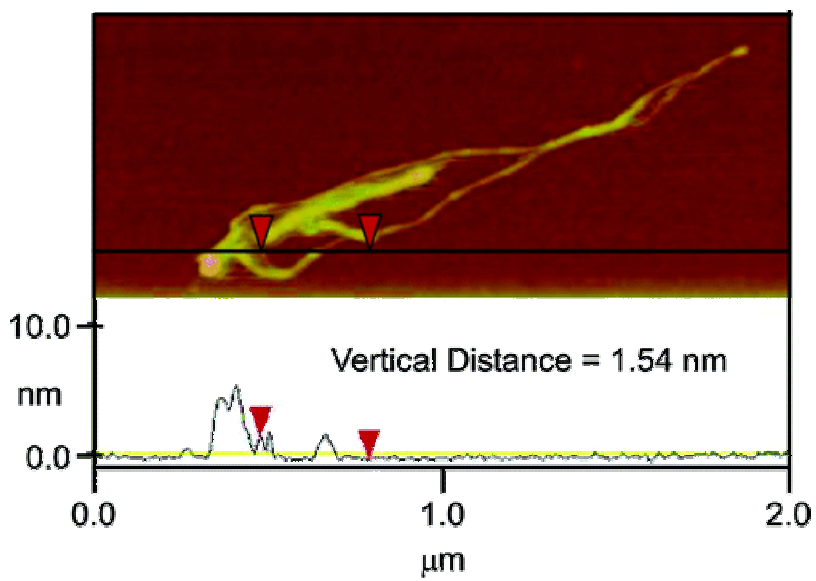


Clusters are Persistent: SANS from Labeled SWCNTs









BJ Bauer, EK Hobbie and ML Becker *Macromolecules,* **2006**, *39(7),* 2637 -2642.

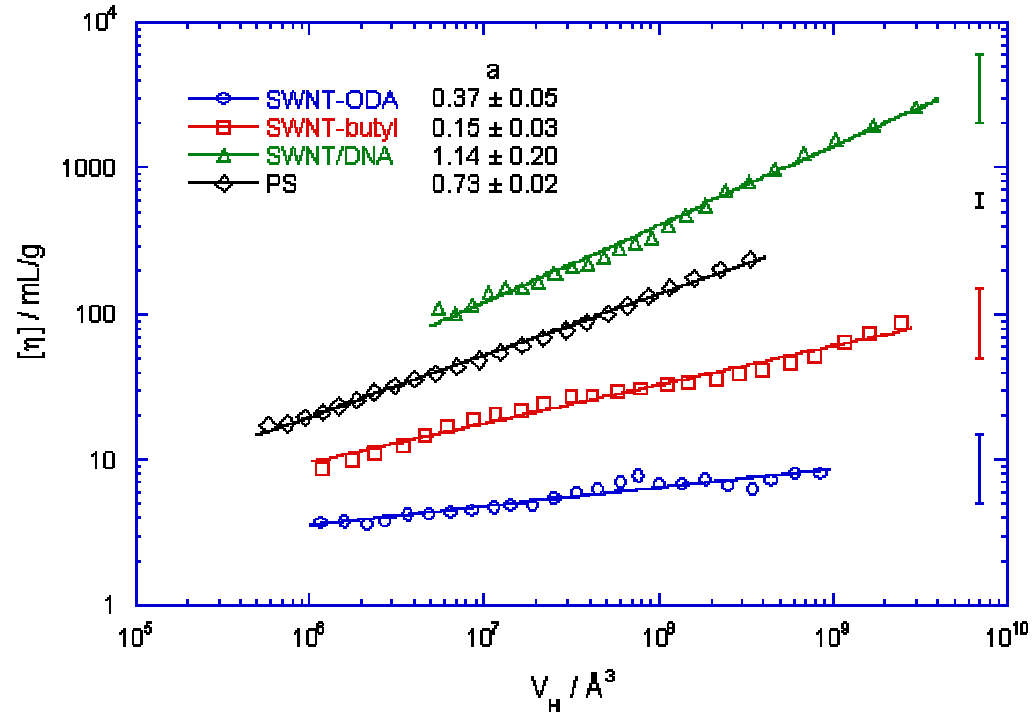
Assessing Dispersion

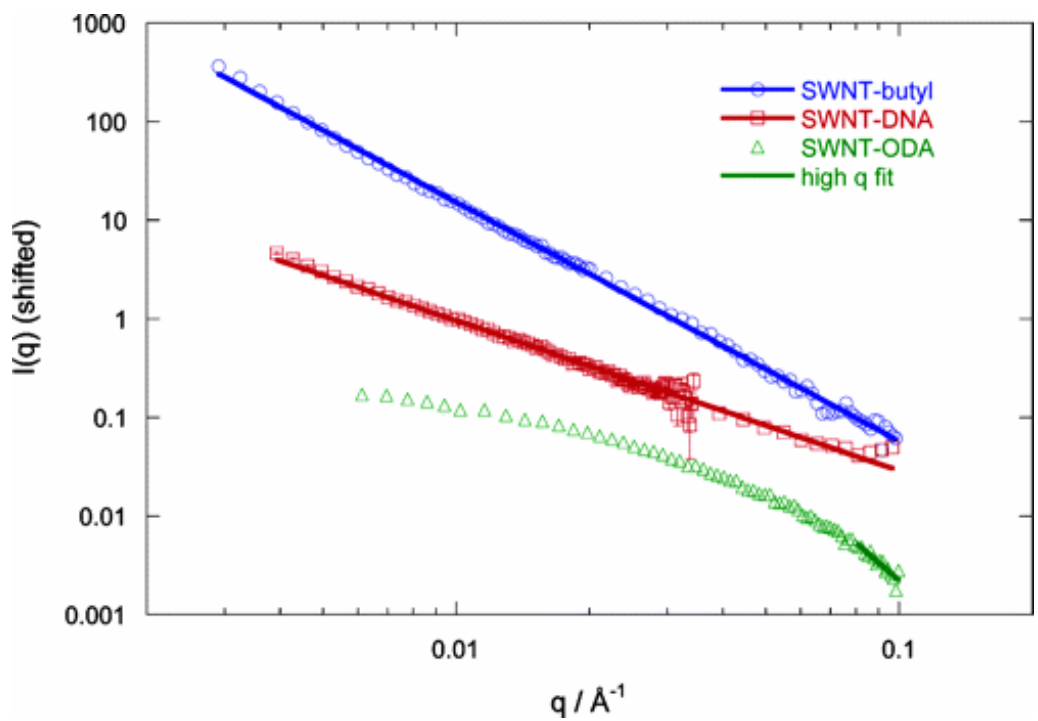
Structure	Viscosity power law $[\eta] \propto M^a$	Scattering power law $I(q) \propto M^{-\alpha}$
Rigid rod	2	1
Flexible rod	0.8 to 2	1 to 1.67
Self-avoiding random walk	0.8	1.67
Gaussian random walk	0.5	2
Branched	< 0.5	2 to 3
Dense sphere	0	4
SWNT - ODA	0.15 ± 0.03	3.9 ± 0.5
SWNT - DNA	1.14 ± 0.20	1.5 ± 0.1
SWNT - butyl	0.37 ± 0.05	2.5 ± 0.2

Dispersion improves significantly after chromatography

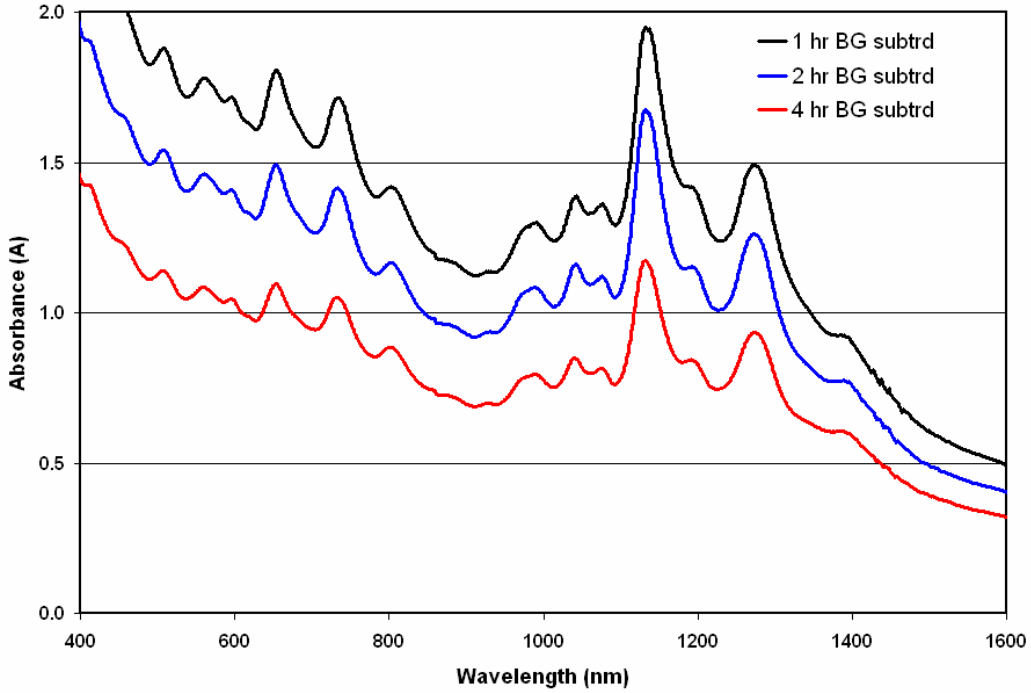
BJ Bauer, et al. *J Phys Chem C*, ASAP

Intrinsic viscosity measured on line by SEC



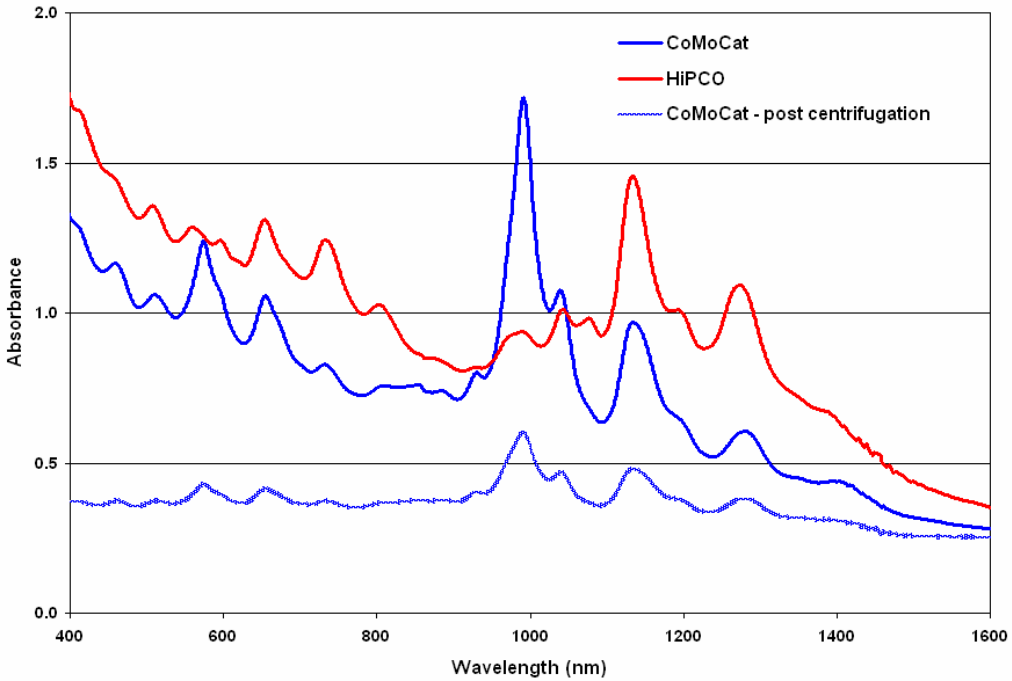


DNA-wrapped SWNT



Increased sonication time reduces nanotube length

Common to all tube types

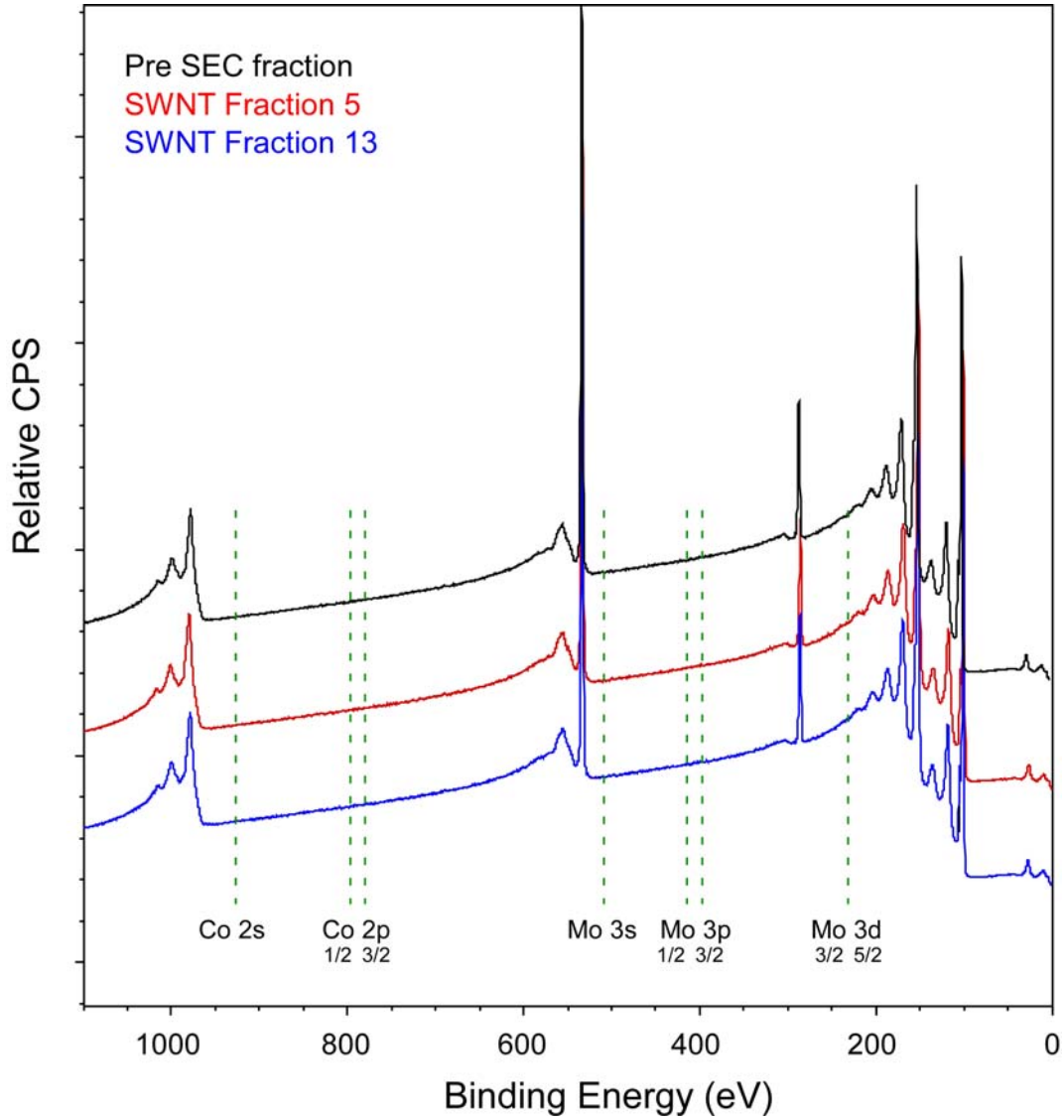


Synthetic method influences tube type and distribution

Centrifugation removes 30-60% of tube mass
Non dispersed fraction

Dispersed tubes are only material useful in processing scale up

What we know…



No detectable catalyst

Well dispersed

Still have tubes

<u>Cell Viability Experiment</u>

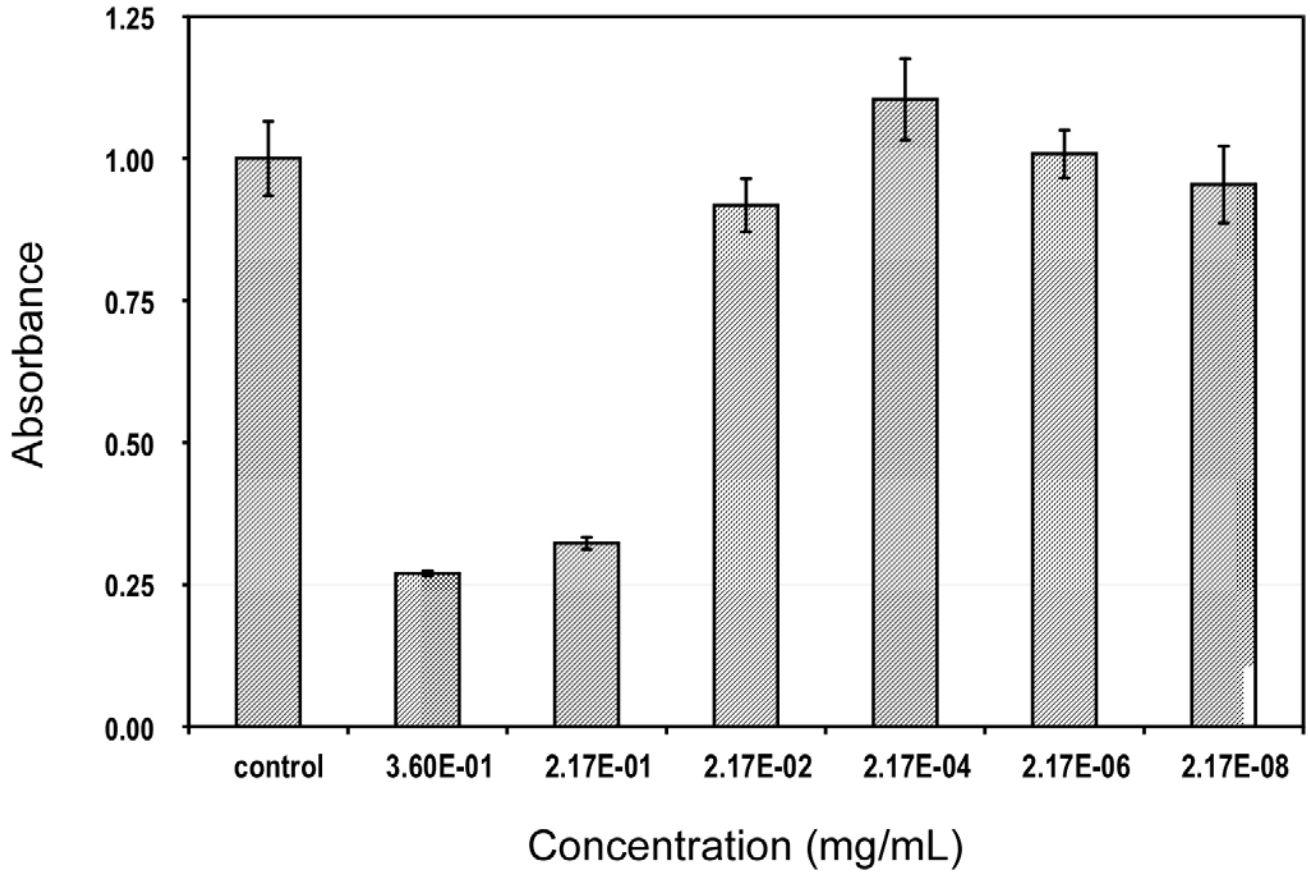
Human Lung Fibroblasts (IMR90)

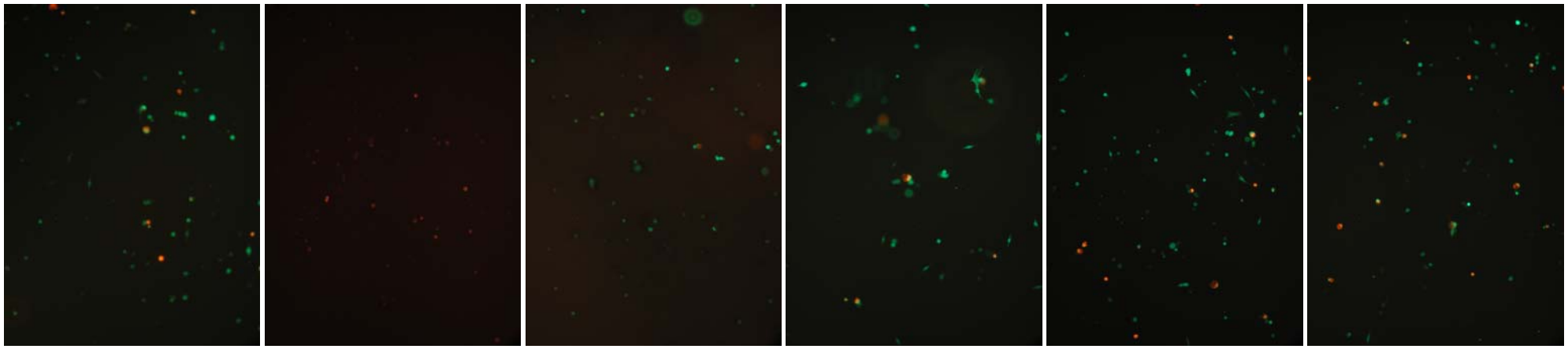
Concentration profile

16 h incubation

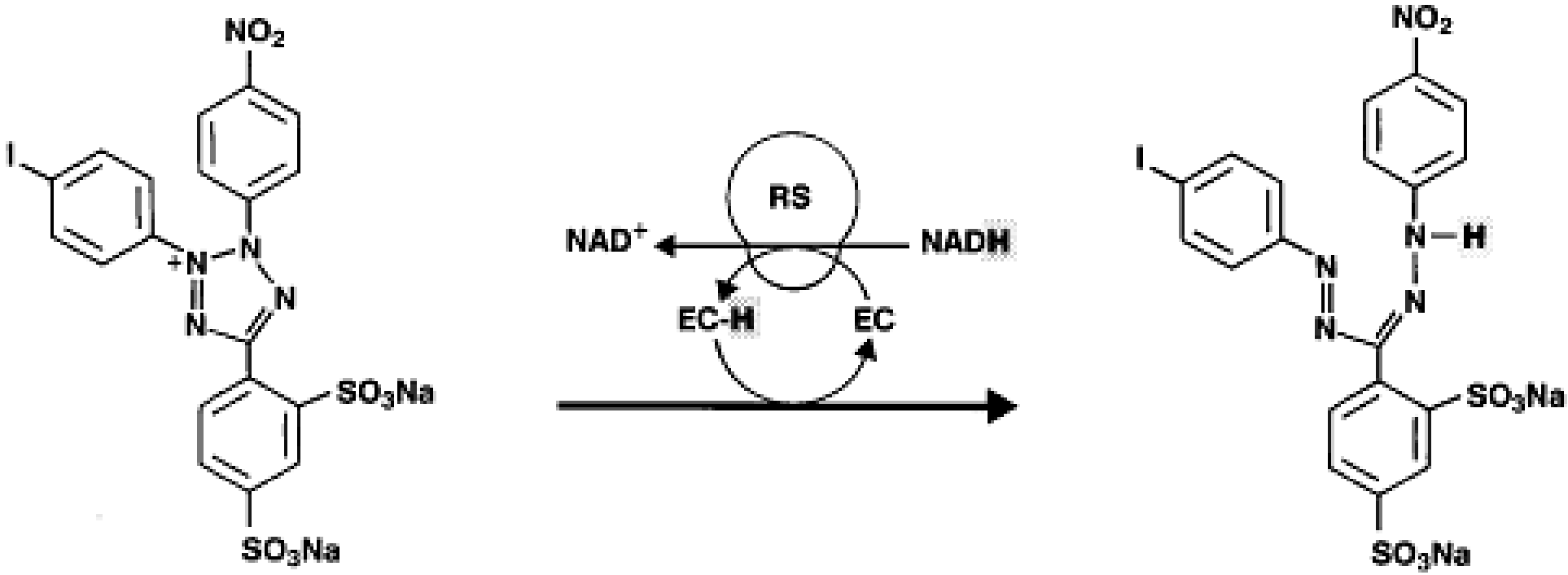
Measure at 405 nm

Simplest bio-assays are very characterization demanding





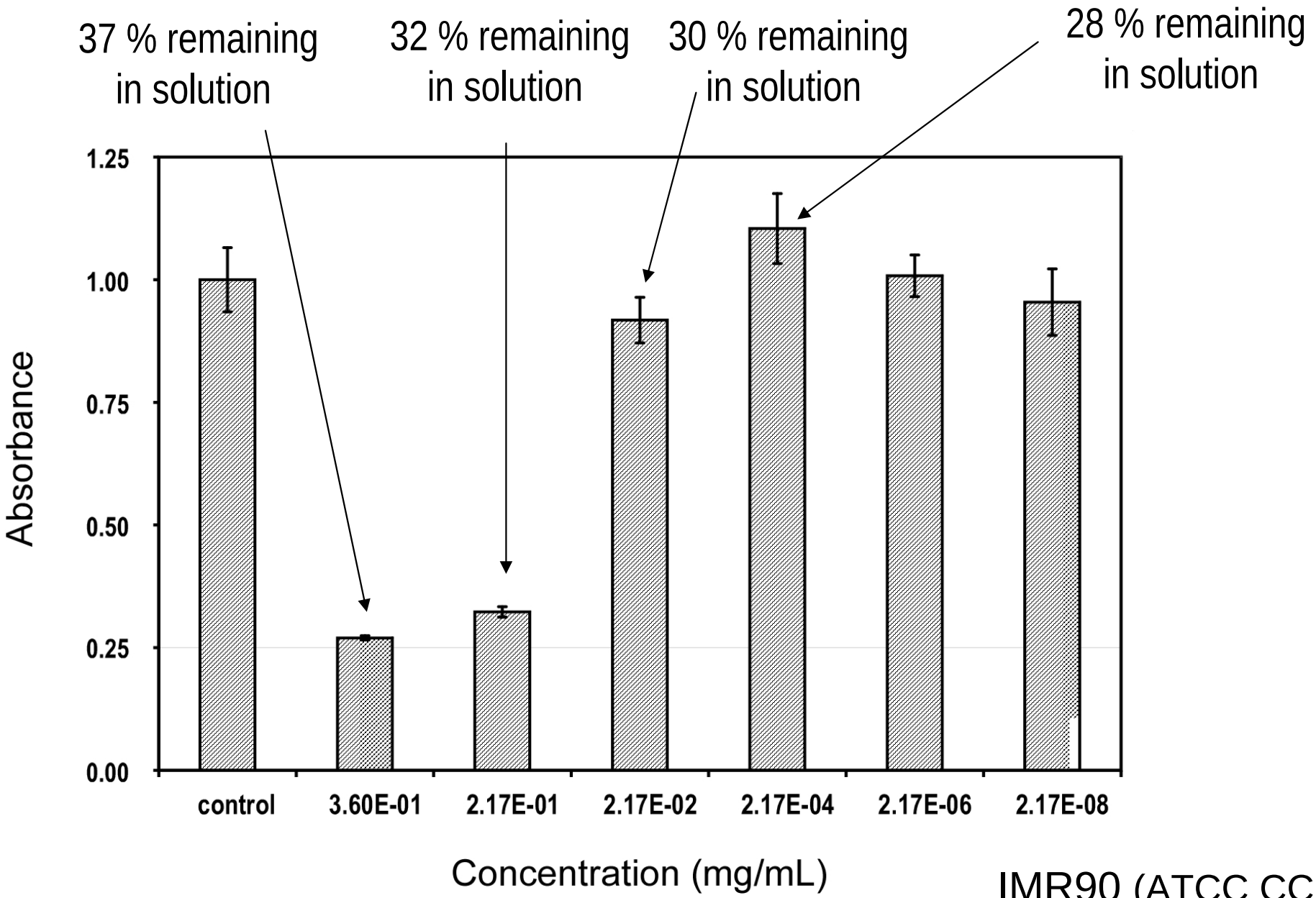
Roche's cell proliferation & cell viability reagent WST-1



WST-1
pink

Formazan
Dark red

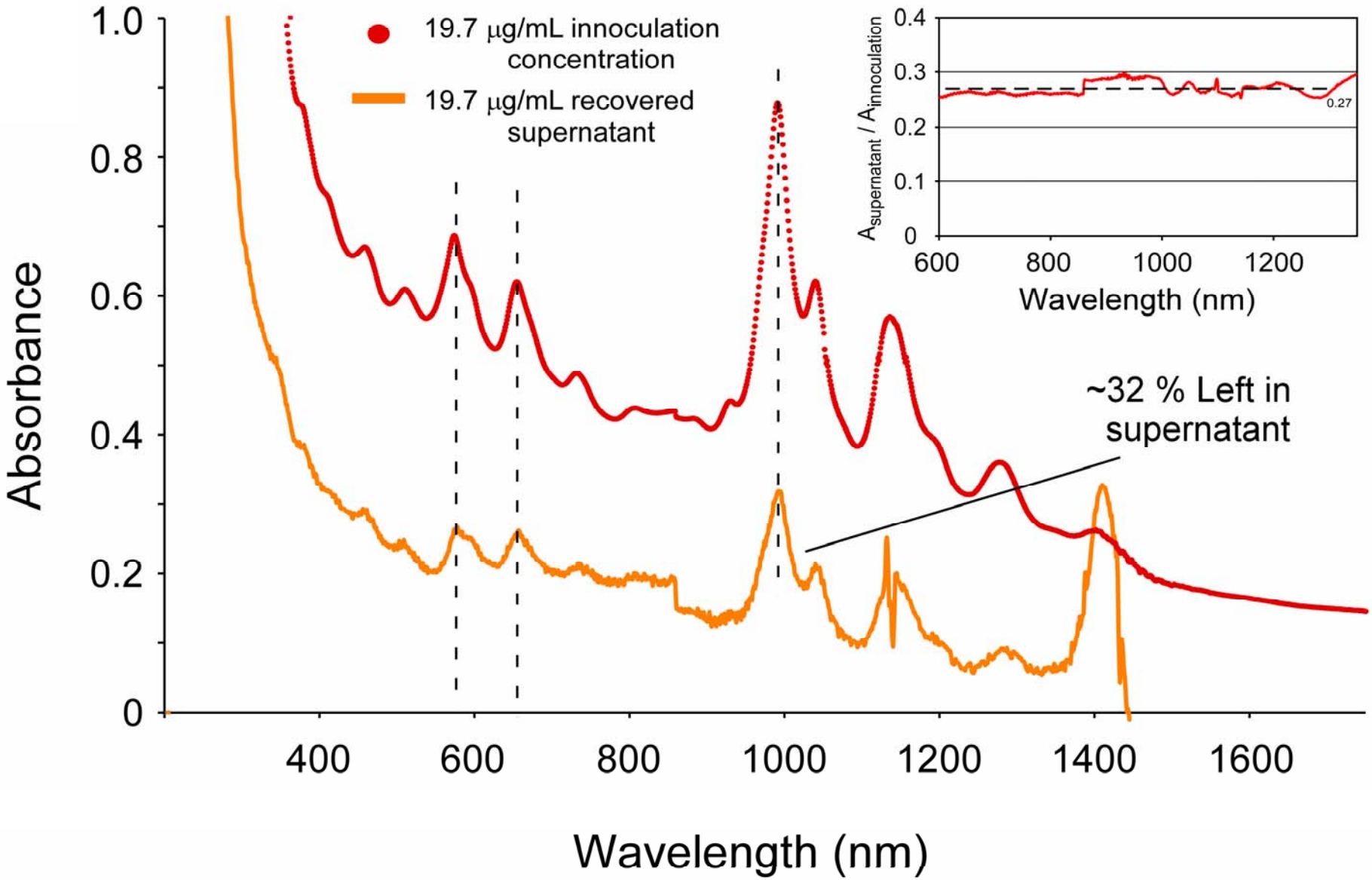
WST-1 tetrazolium salt is reduced to formazan by cellular dehydrogenases generating a deep red colored formazan that is measured at 405 nm and is directly correlated to cell number.



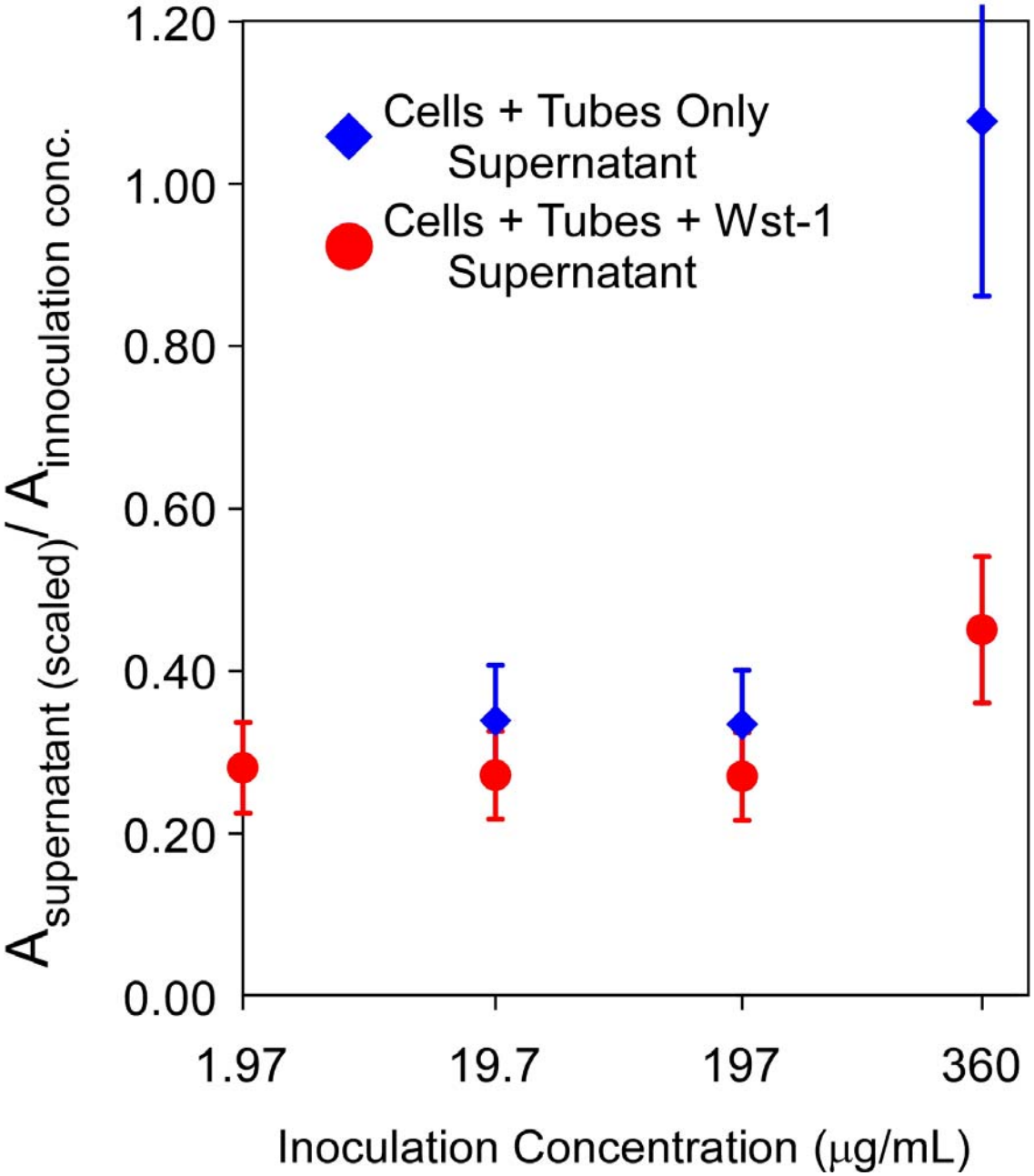
IMR90 (ATCC CCL-186)
Primary human fibroblast
derived from fetal lung tissue

~ 30 % of tubes remaining in solution at each concentration
–> length dependent uptake ?

No chirality dependence - length dependent uptake ?

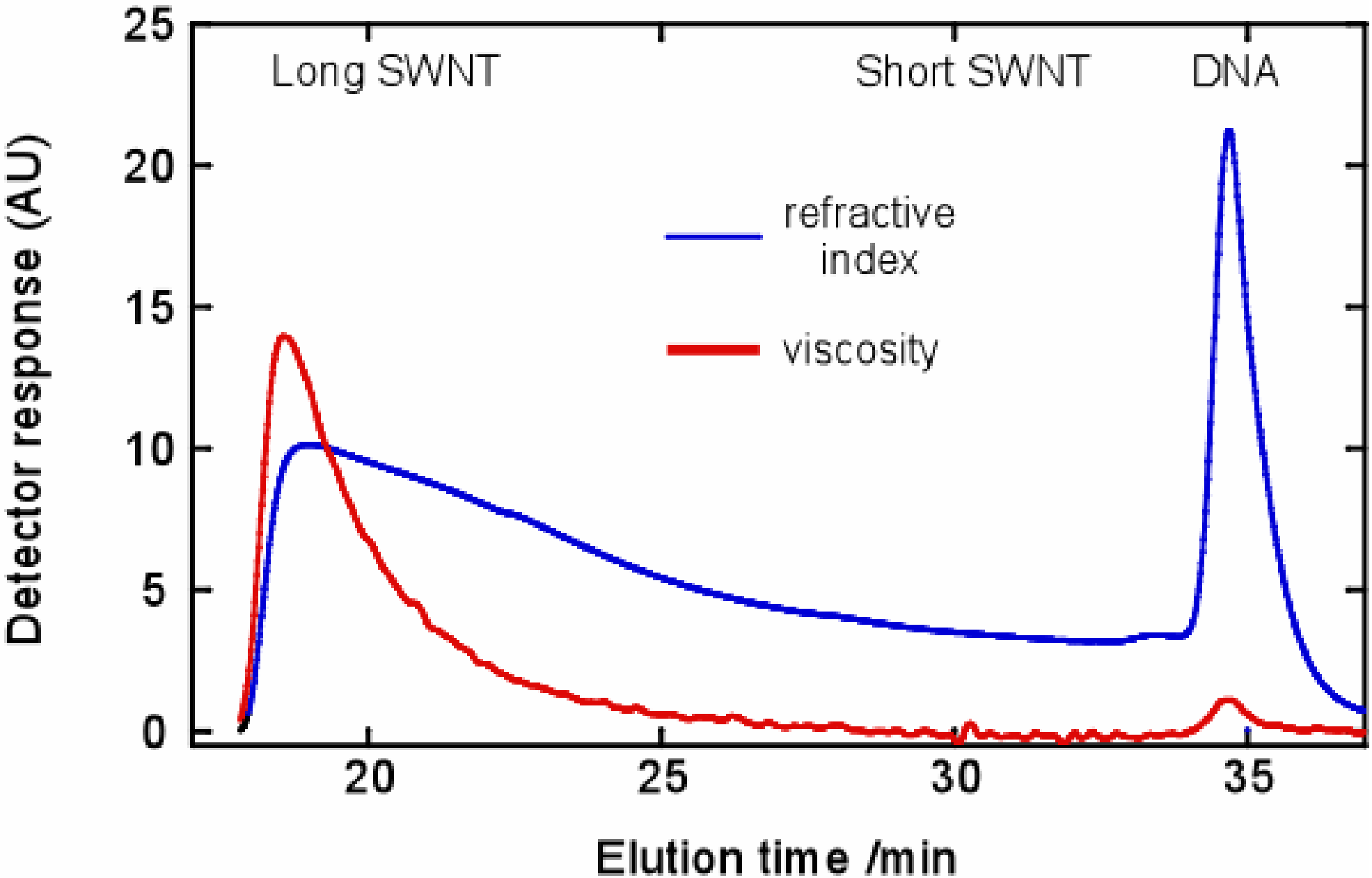


A closer look at what remains…



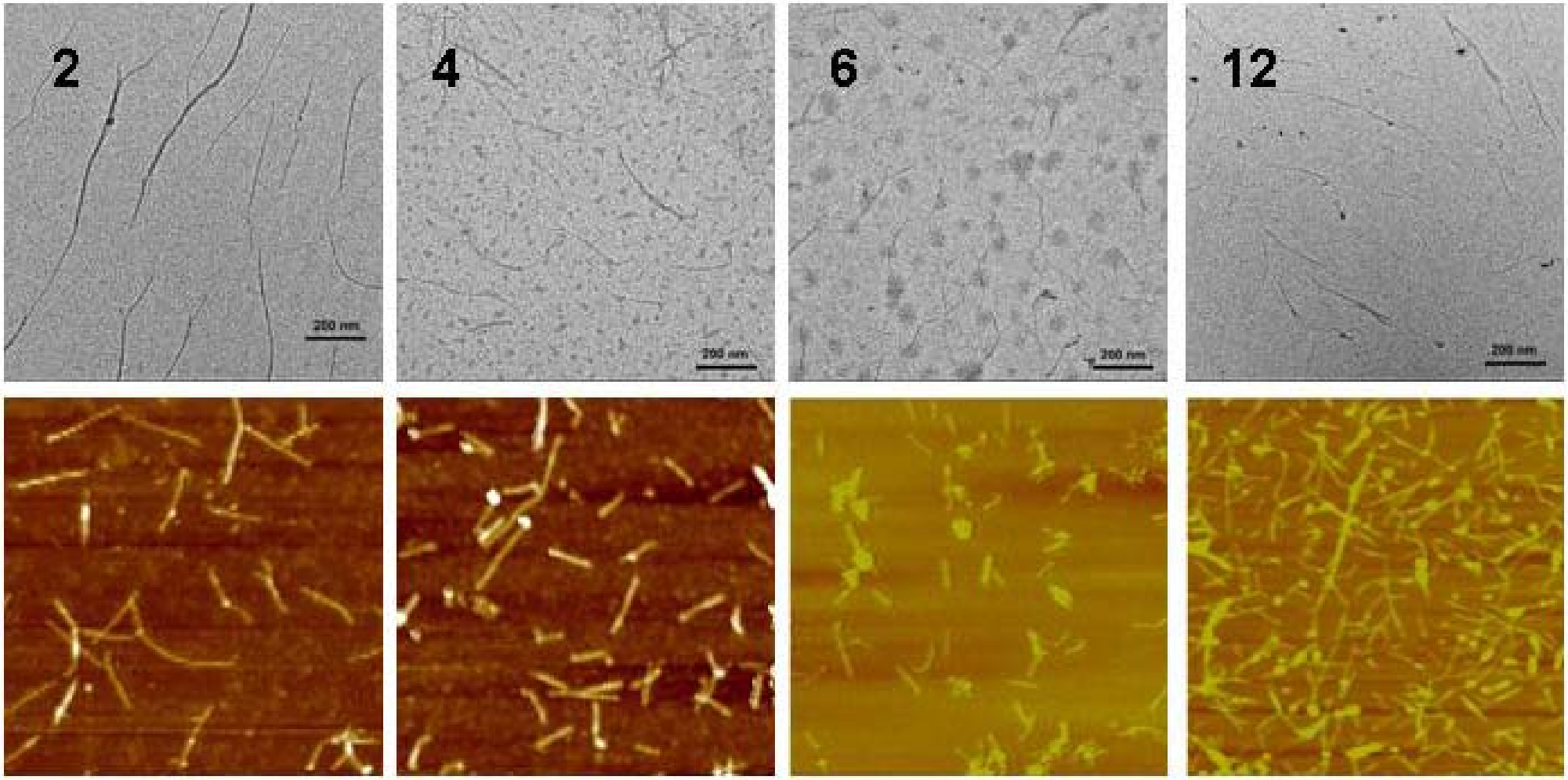
Cells "swamped" at the highest concentrations and cease metabolic activity

SEC Separation by SWNT Lengths



Collecting intermittent fractions affords length separation of DNA-wrapped SWCNTs

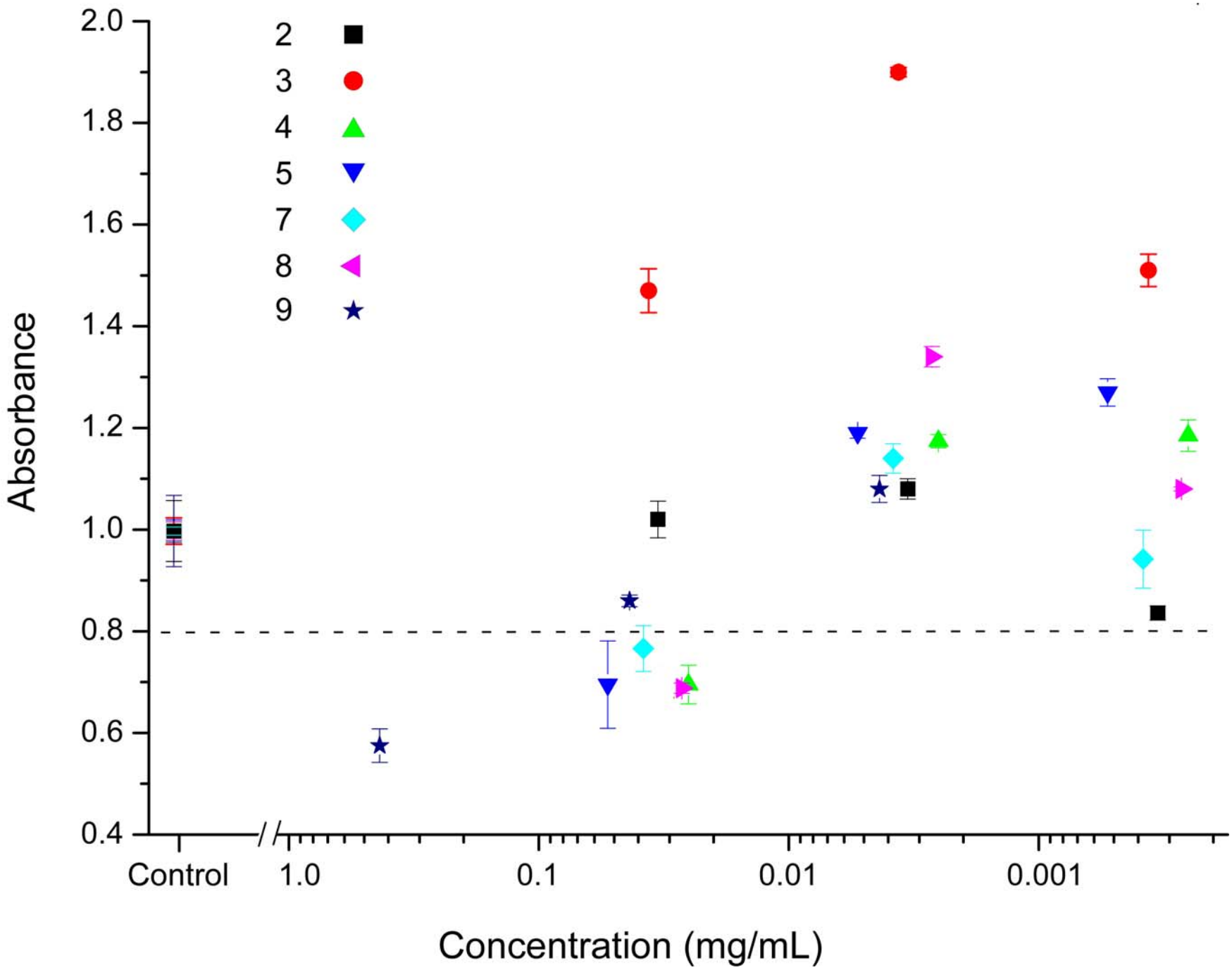
Thorough Characterization via TEM, AFM, DLS

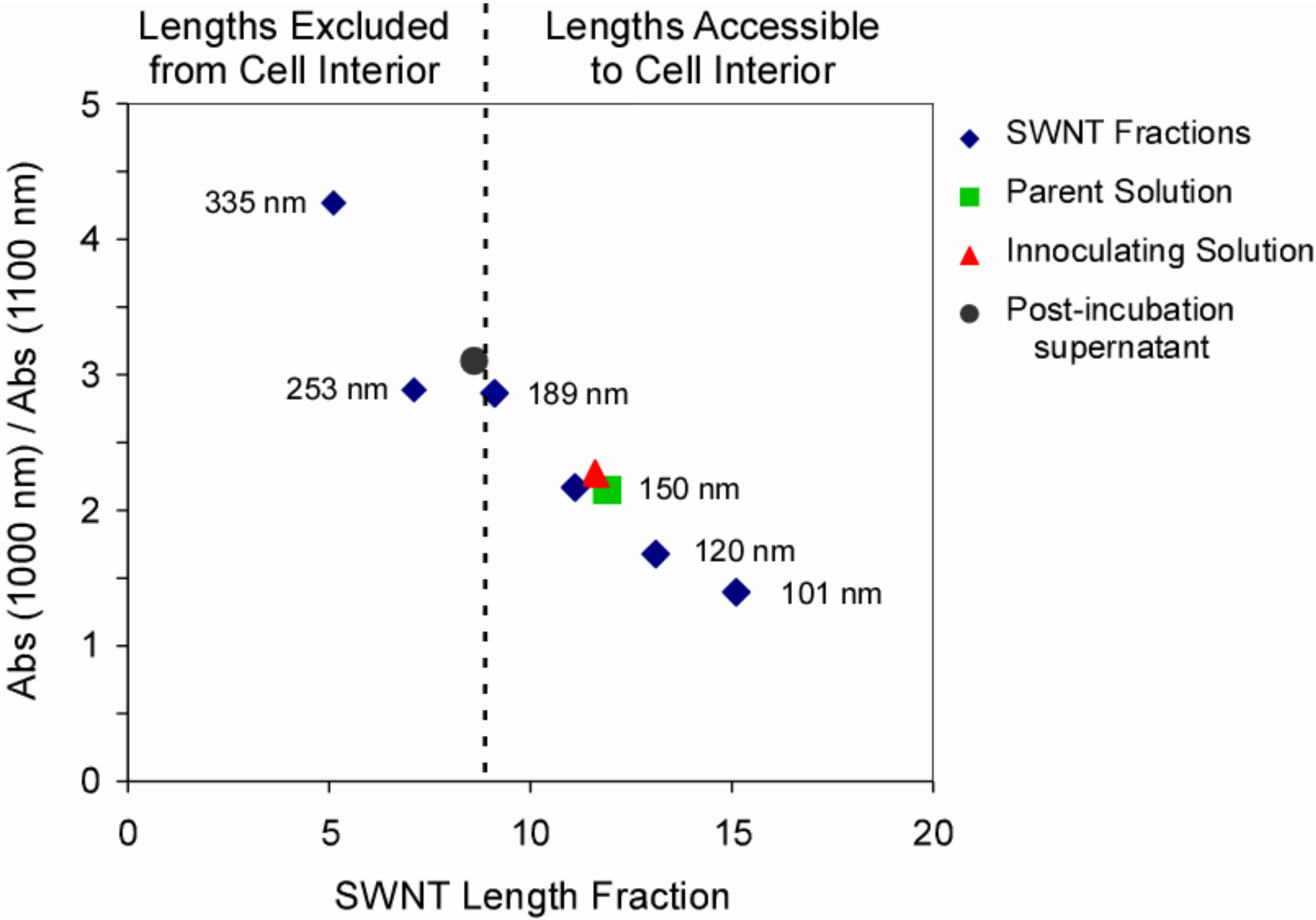


Characterization Data for Individual Length Fractions

Sample fraction	Rg (SEC) (nm)	Length (SEC) (nm)	Length (AFM) (nm)	Concentration (mg/mL)
1	113.5 ± 5.1	393 ± 18	-	0.031
2	96.8 ± 7.9	335 ± 27	367 ± 61	0.167
3	72.9 ± 7.4	253 ± 26	303 ± 11	0.180
4	54.6 ± 5.0	189 ± 17	210 ± 48	0.126
5	42.4 ± 3.4	150 ± 12	149 ± 43	0.266
6	34.7 ± 2.6	120 ± 9	138 ± 60	0.119
7	29.2 ± 2.0	101 ± 7	76 ± 27	0.191
8	25.8 ± 1.4	89 ± 5	-	0.134
9			distribution	

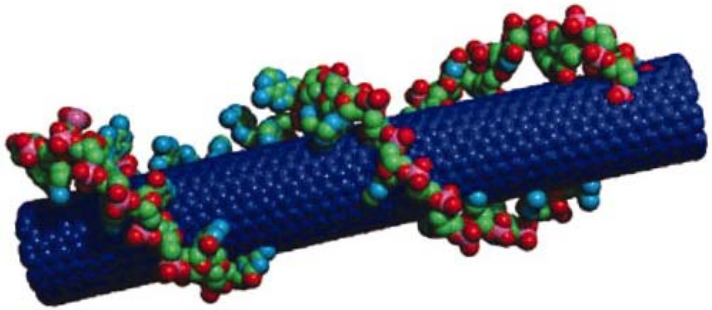
Length Dependent Uptake from Following 16 h Incubation



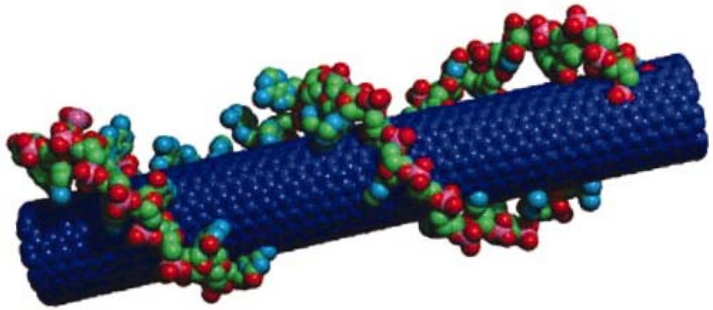

Lengths Excluded from Cell Interior
Lengths Accessible to Cell Interior
Abs (1000 nm) / Abs (1100 nm)
SWNT Length Fraction
0
1
2
3
4
5
0
5
10
15
20
335 nm
253 nm
189 nm
150 nm
120 nm
101 nm
SWNT Fractions
Parent Solution
Innoculating Solution
Post-incubation supernatant

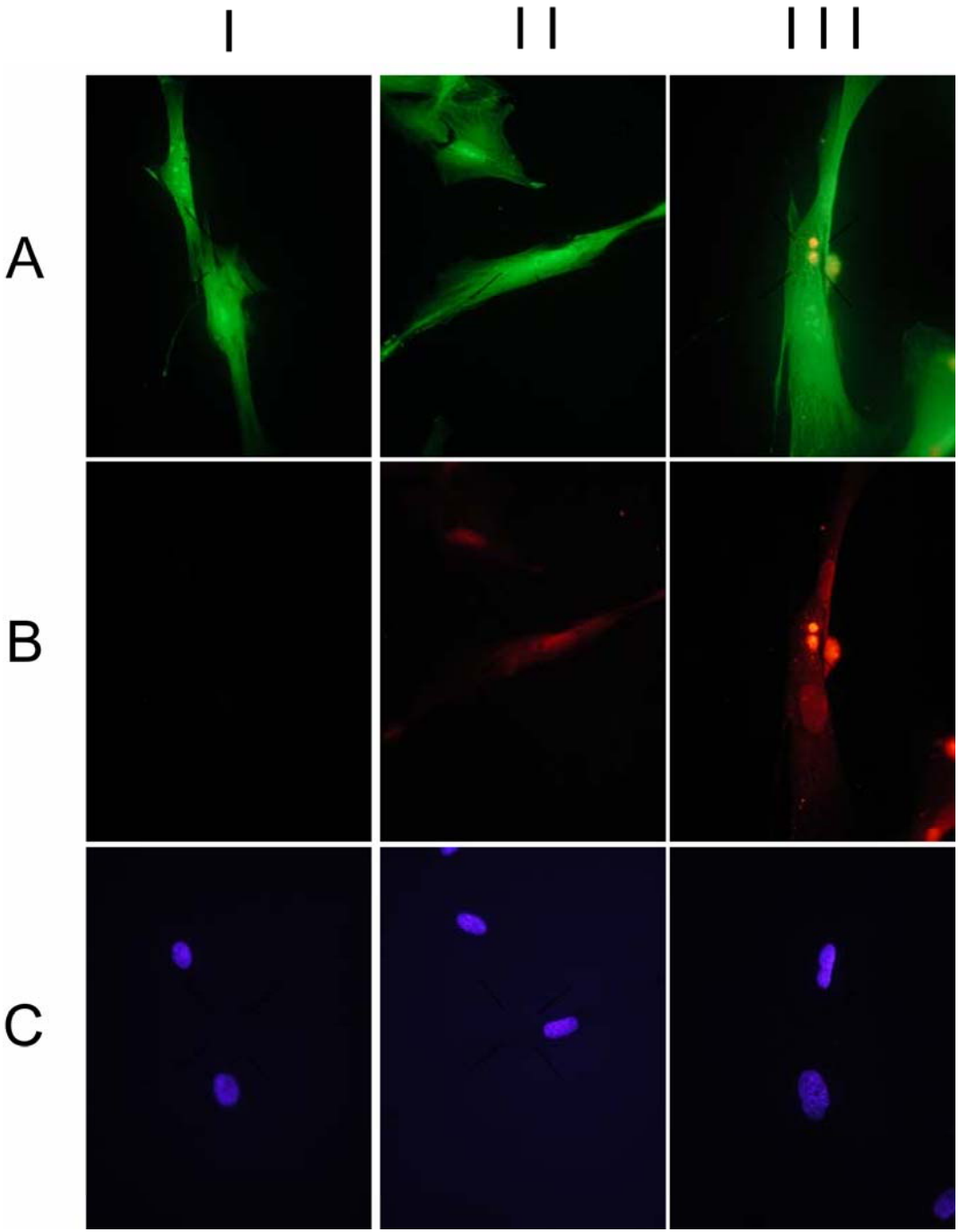
Competitive uptake of labeled DNA wrapped SWNTs

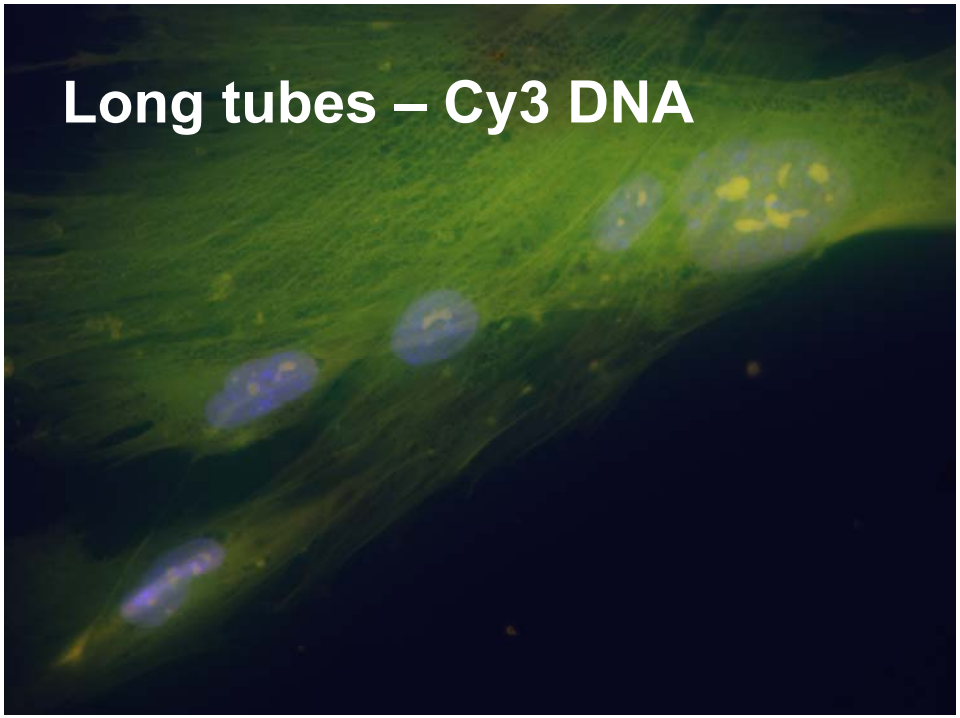
Long tubes – Cy3 DNA

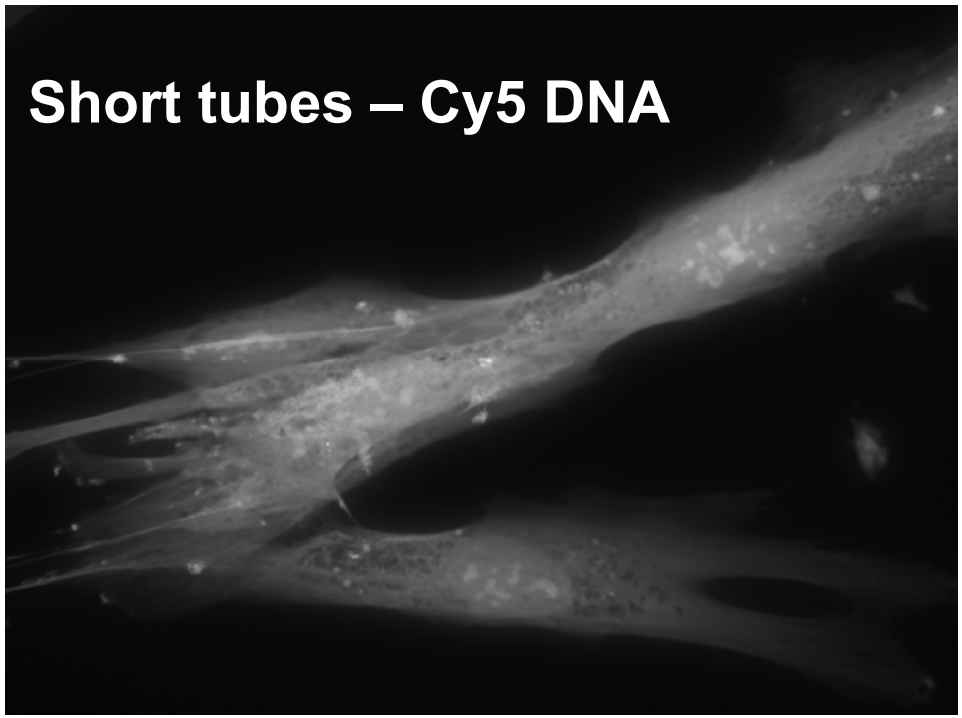


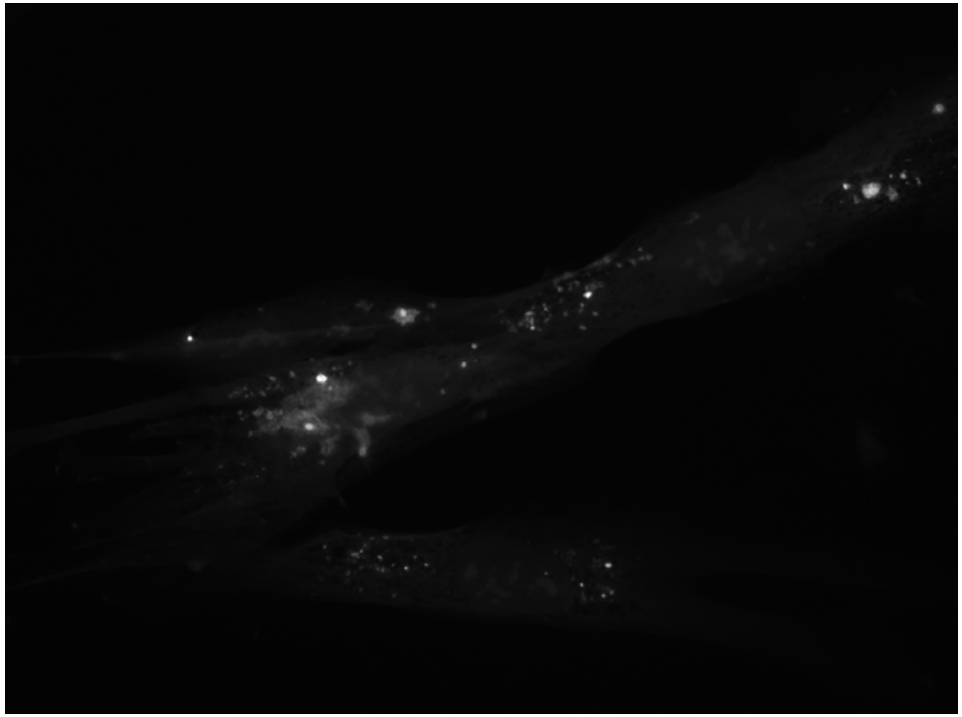
Short tubes – Cy5 DNA



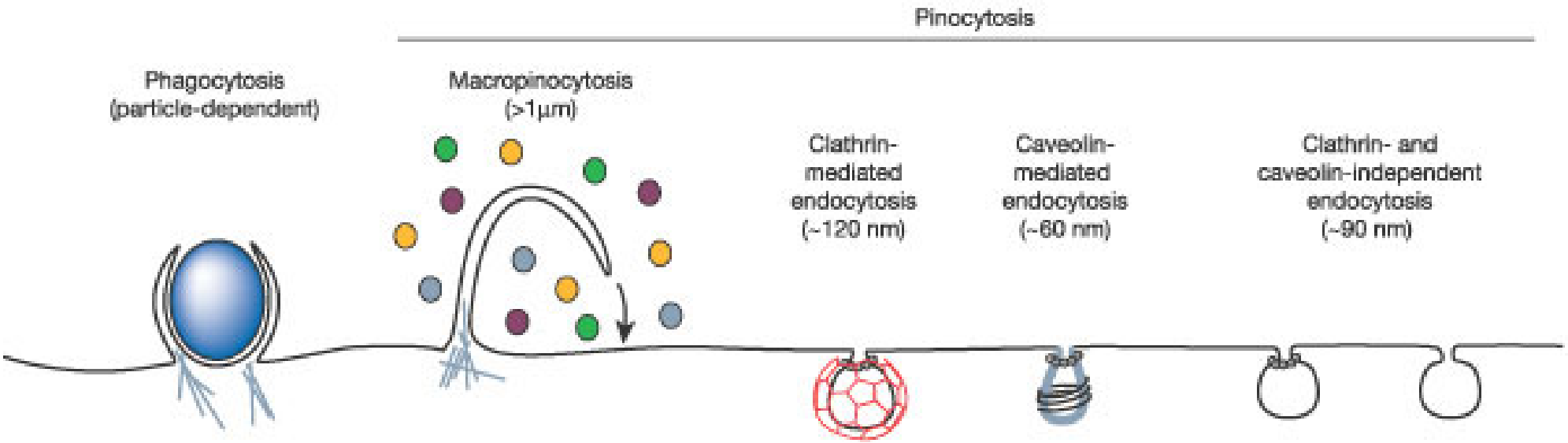



Long tubes – Cy3 DNA


Short tubes – Cy5 DNA



These are the known mechanisms of cell membrane transport



They are coordinated surface-mediated self-assembly interactions with significant consequences

Permeability through capillaries once they enter the bloodstream - biodistribution

Intracellular transport by traditional and non-endocytotic pathways - toxicity

Retention in the liver, lungs and kidneys - clearance

Conclusions

Small physico-chemical increments have significant bio-implications

Poorly characterized materials are useless for bio measurements

Simple measurements powerful if inputs are known

Acknowledgement

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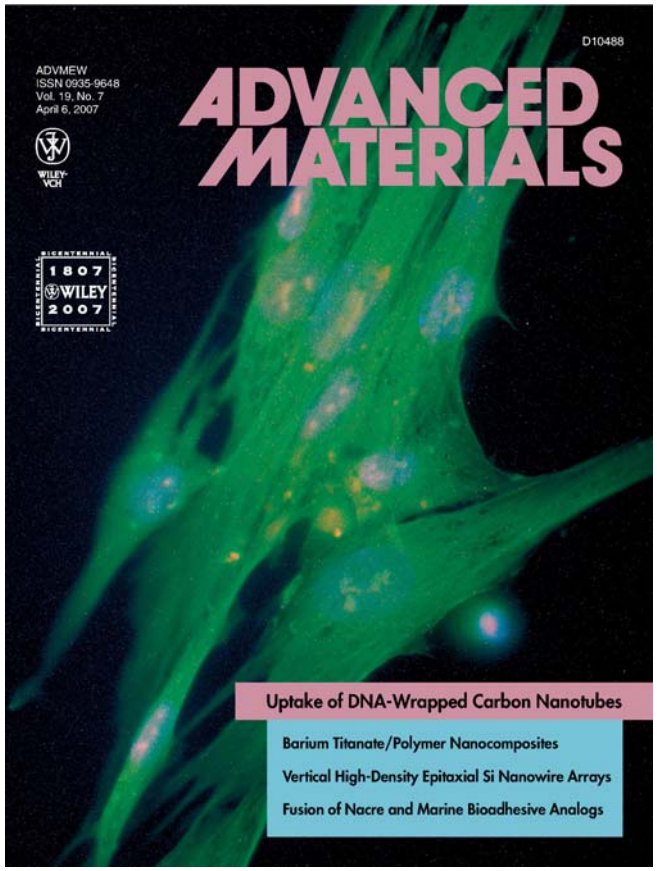

Where we were 2 Years ago…

Two workshops held at NIST: 2003, 2005

Over 150 attendees from industry, academia, and government

- How does one accurately measure the quality of a nanotube batch?
  - Species quantification critical to enable material optimization
  - Product developers need assurance that incoming materials are optimized
- How does one ensure sufficient dispersion for next-stage processing?
  - Propensity for agglomeration poses formidable challenges for processing
  - Nano-dispersion (isolation of individual tubes) critical for high-end nanofab-electronics, opto-electronics, and sensors

National Aeronautics and Space Administration
Lyndon B. Johnson Space Center

NIST
National Institute of Standards and Technology
Technology Administration
U.S. Department of Commerce

2nd Joint Workshop on Measurement Issues in Single Wall Carbon Nanotubes: Purity and Dispersion Part II

Co-Sponsored by NASA and NIST

January 26-28, 2005
NIST, Gaithersburg, MD, USA

WORKSHOP GOALS

The first NASA-NIST "Measurement Issues" workshop was held in May 2003 and focused on challenges in purity and dispersion measurements for single wall carbon nanotubes (SWCNTs). A wide range of analysis methods were discussed, including TGA, TEM, SEM, Raman, etc. Participants agreed that protocols were sorely needed, although many details were, at that time, still incomplete. A summary of this workshop can be found at www.msel.nist.gov.

Since that first workshop, several other conferences have also stressed the importance of SWCNT standards. Recent publications have shown continued and substantial advancements in purity analysis, including separation of metallic and semiconducting tubes. In addition, the SWCNT community remains focused on "debundling" and dispersing nanotubes to form advanced composites, while also separating and utilizing individual tubes in novel electronic and biological applications.

The present workshop will bring together leading nanotechnology researchers to discuss, and ultimately agree, on standard protocols for measuring the purity and dispersion of SWCNTs. Participation by a diverse group of industrial, government, and academic researchers is solicited to ensure comprehensive discussion from an array of viewpoints and application areas. The primary goal is to jointly overcome key measurement issues that limit commercialization, thereby enabling rapid growth of this high-impact technology area.

WHY NOW?

Based on the continued and rapid growth of SWCNTs, we feel that it is time to revisit critical purity and dispersion issues, as well as to join together to address future measurement challenges of broad interest. The goal of this second workshop will be collaborative development of "Recommended Practice Guides" to assist researchers across the field in SWCNT characterization. In addition, new measurement-related topics will be introduced, including the availability of methods for isolating and measuring the physical and functional properties of individual nanotubes and the need for, and development of, reference standards.

MEETING FORMAT

- Invited review talks
- Contributed papers & posters
- Open-forum discussions
- Breakouts for in-depth discussion
- Final planning session on next steps and goals

KEY QUESTIONS

- What is state-of-the-art in purity analysis? What are the limitations? What is needed in the future?
- What methods are available to debundle and disperse nanotubes? How is dispersion quality measured? Is this sufficient for application development?
- What techniques are used to measure size, chirality, and functional properties? What improvements are needed?
- What measurement priorities should NIST, NASA, and the nanotube research community work on together?