Nanomaterial Cytotoxicity

Silvia Lucatero

Allan Hancock College

Physiology

Mentor: Dr. Won Hyuk Suh

P. I.: Prof. Galen D. Stucky

Chemistry Department, UCSB

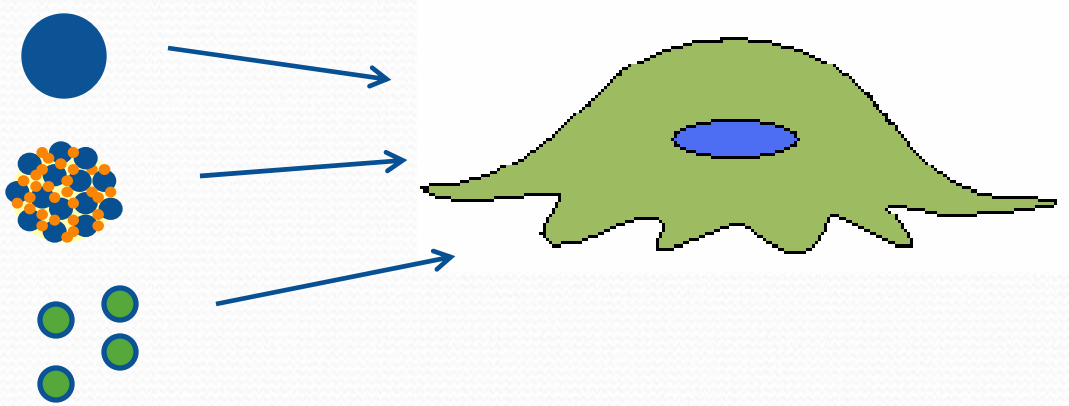
Funding: Environmental Protection Agency (EPA)

Otis Williams Fellowship

Nanostructured Materials Dangerous?

- Nanostructured materials are new technology
- Small nature can influence cell behavior

How do cells respond and get affected?



- Sizes
- Surface chemistry
- Contents

Research Objectives

- **Optimized screening protocol establishment for synthetic nanomaterials (i.e. TiO_2, SiO_2, Fe_2O_3, Carbon, other Metal Oxide) (Synthesis by Mr. Bedford)**

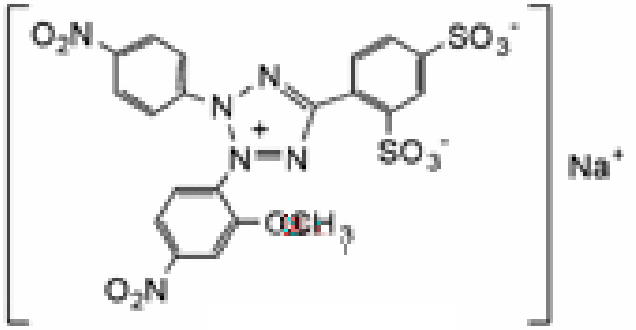
- **Methodologies**
  - **Cell viability/toxicity, nucleic acid, protein, metabolite content analysis**
  - **Water soluble tetrazolium salt (WST)**
  - **Measurement platform: microplate reading**

- **Proceed with cell culture experiments**

  in vitro → ***in vivo*** → **Clinical Studies**

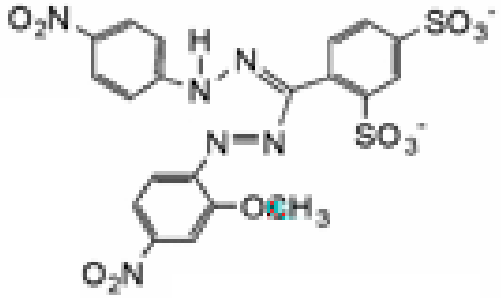
Water Soluble Tetrazolium (WST) Assay

- Colorimetric assay used to determine cytotoxicity
- WST-8 reduced in living cells to WST-8 Formazan
- Amount of formazan produced corresponds to number of living cells



WST-8
slightly yellow

Cellular reduction



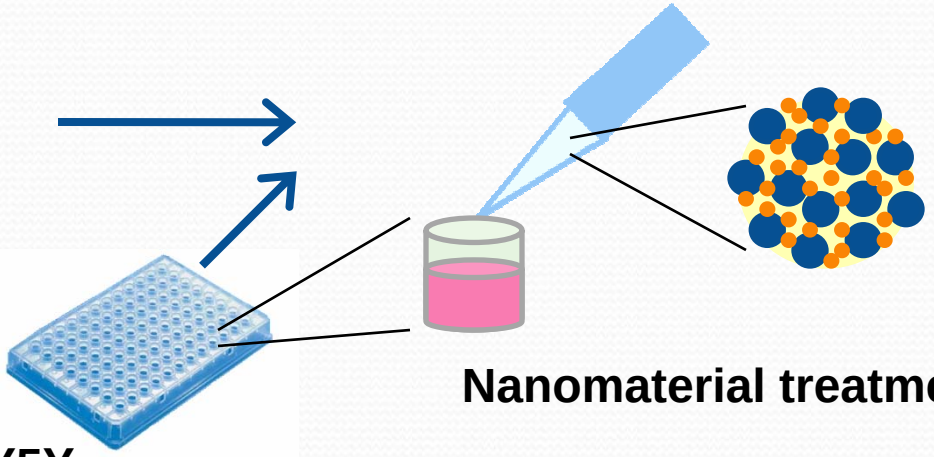
WST-8 Formazan
orange dye

O.D. at 450 nm

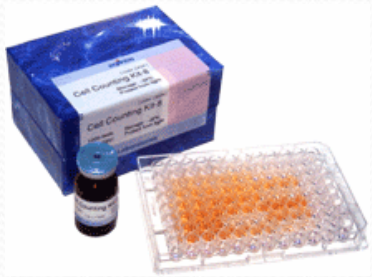
WST Assay Procedure

BV2 microglia

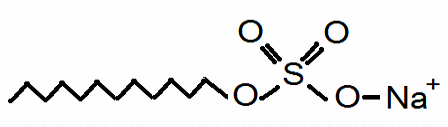
SH-SY5Y neuroblastoma



Nanomaterial treatment



WST-8 incubation



sodium dodecyl sulfate (SDS) to stop the reaction

Microplate reader determines the absorbance of each well

Compare results with control

Dojindo, Inc., www.dojindo.com, Thomas Industrial Network,

Slide 5

O4

Microplate Readers (also known as Plate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. Sample reactions can be (assayed) in 6-1536 well format microtiter plates. In most cases, a high-intensity lamp passes light to the microtiter well and the light emitted by the reaction happening in the microplate well is quantified by a detector. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. The first microplate readers available were filter based while modern day readers are tunable(monochromator based) enabling use of any fluorophore and chromophore, allowing assay flexibility as needed in the laboratory. Current day plate readers come with software tools for data analysis, automation, GxP tools, and LIMS capabilities.

Microplate Detection may used for:

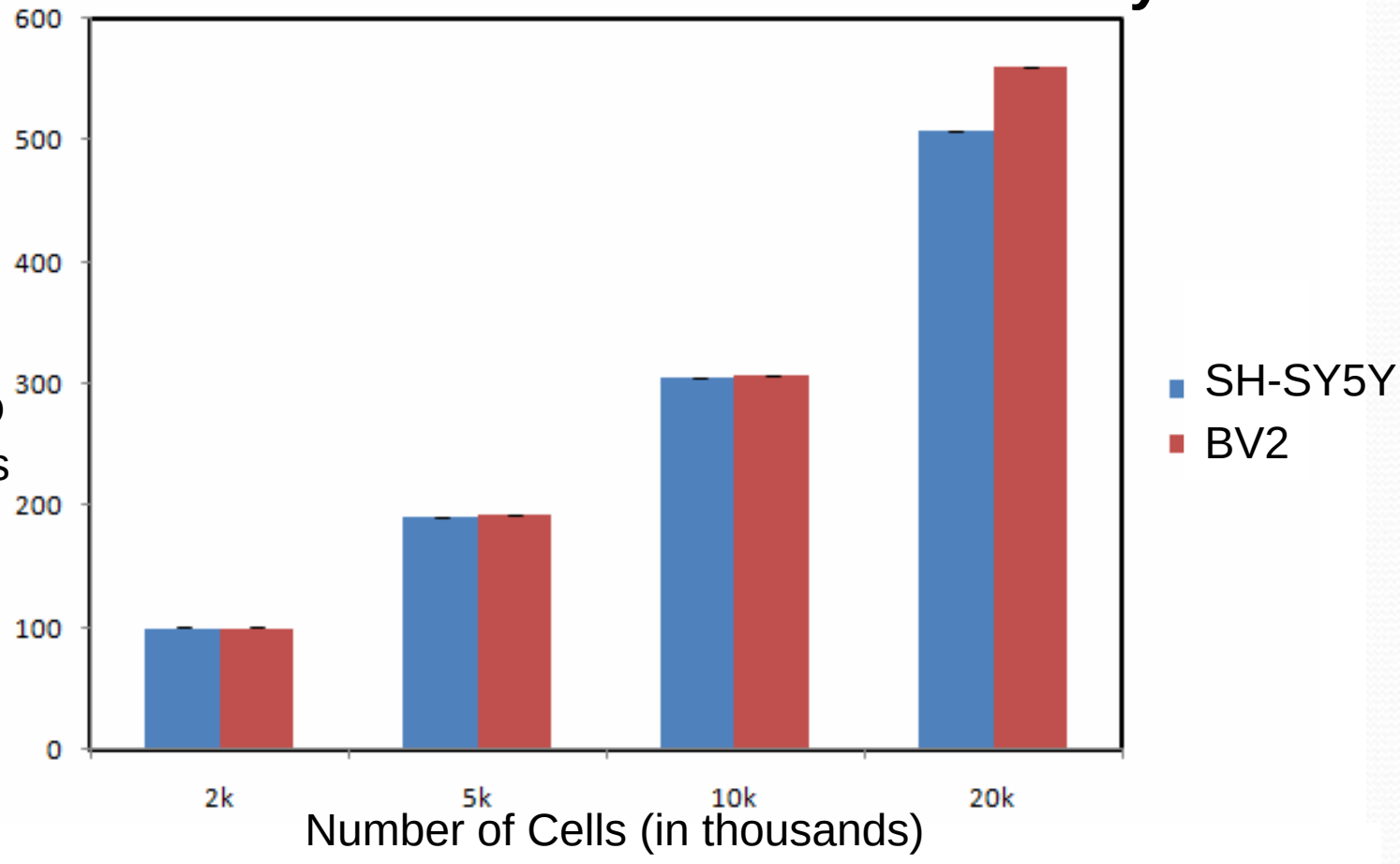
ELISAs
Protein and cell growth assays
Nucleic acid quantitation
Molecular interactions
Enzyme activity
Cell toxicity, proliferation, and viability
ATP quantification
Immunoassays

Owner, 7/25/2008

Cell Counting using WST-8

- **Optical density (OD) measurement at 450 nm**
- **Cell incubation (stabilization) time required: 12-24 hrs**
- **Cell concentration affects OD value: viability**

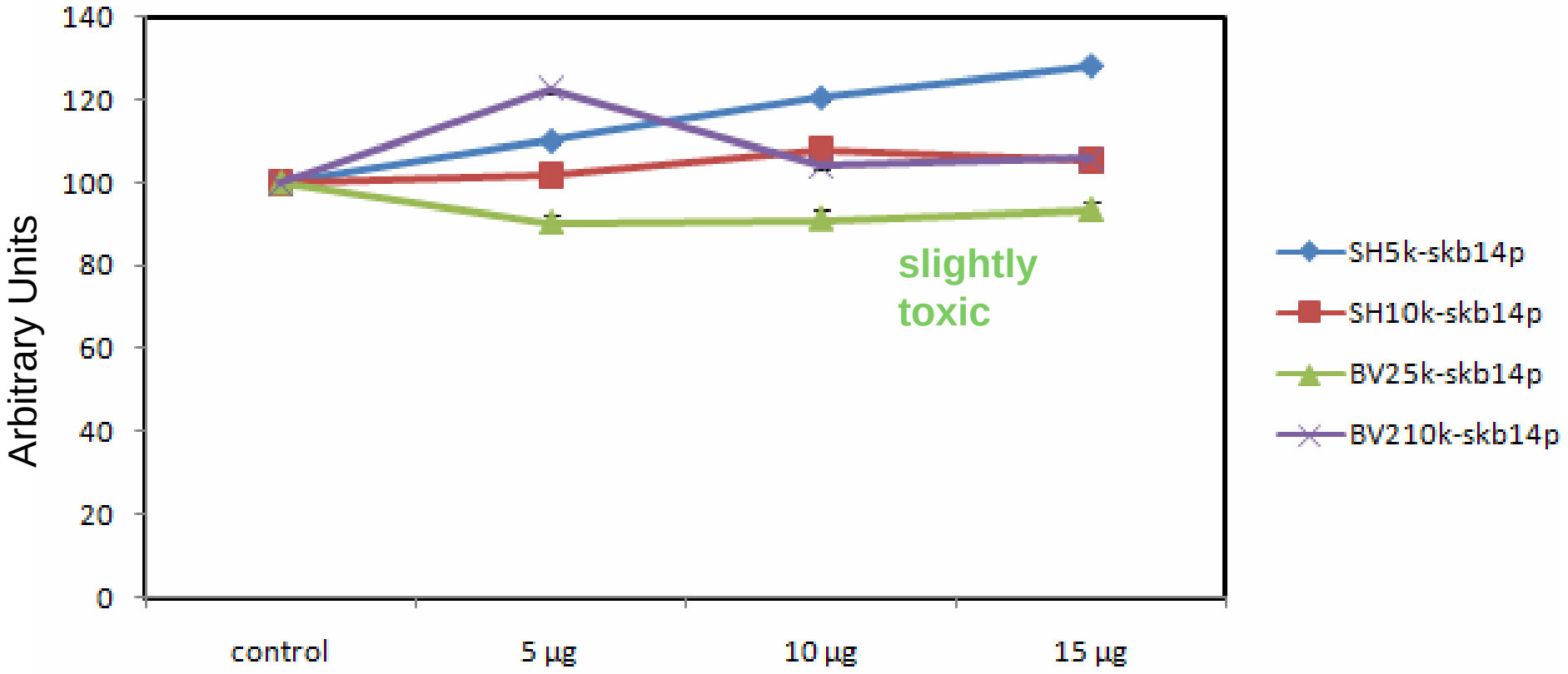
y-axis: OD for 2k cells were set to 100%



O1

Cytotoxicity of Particles

Conditions: plating done 1 day prior, 5 hour particle treatment, OD after 2.5 h



x-axis: amount of particles added into well

- **SH#k = # x 1000 cells SH-SY5Y, BV2#k = # x 1000 cells**
- **skb14p = test particle synthesized from the Stucky group**

O1 Optical density, or OD, is the absorbance per unit length, i.e., the absorbance divided by the thickness of the sample, although it is sometimes used as a synonym for the absorbance with a base-10 logarithm.

In optics, density is a unitless measure of the transmittance of an optical element for a given length at a given wavelength λ:[1]

O = the per-unit opacity
T = the per-unit transmittance
I0 = the intensity of the incident light beam
I = the intensity of the transmitted light beam

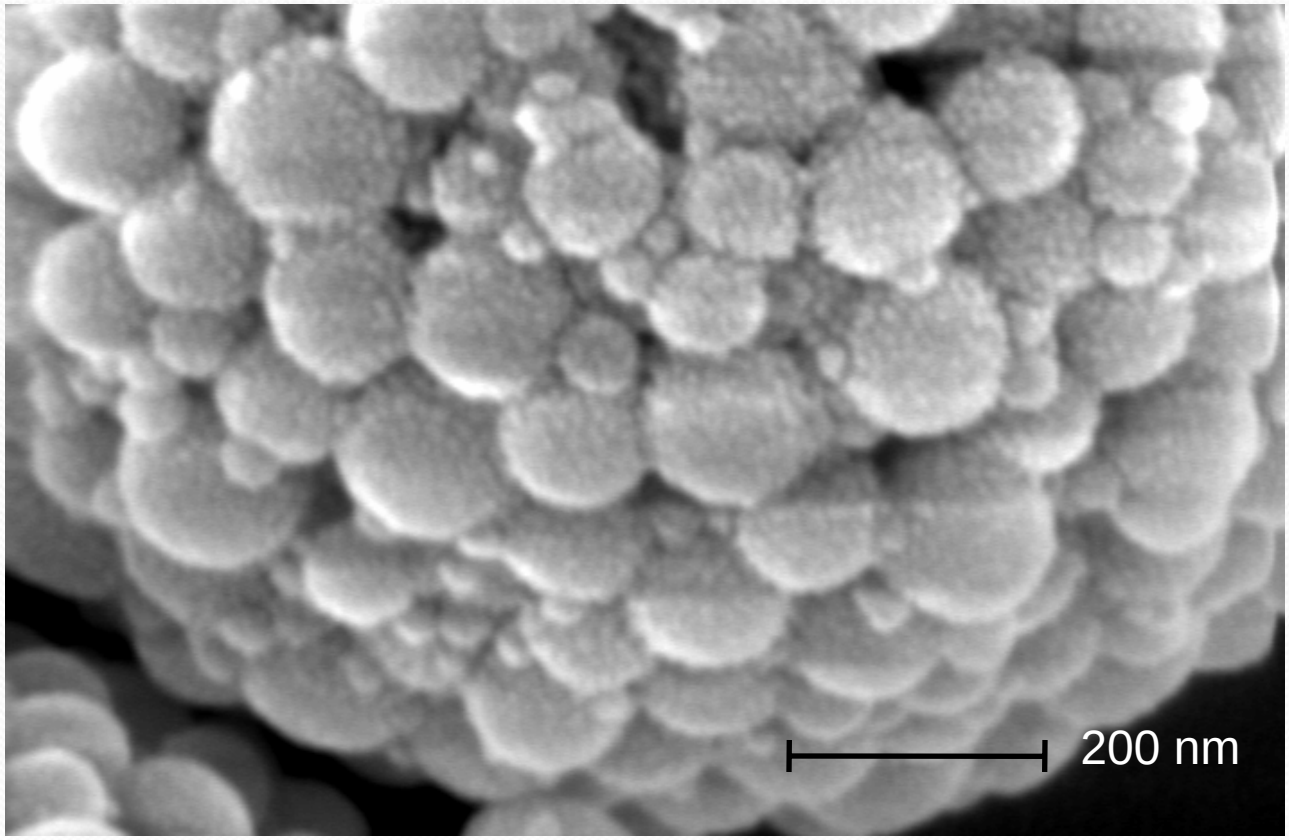
The higher the optical density, the lower the transmittance.

Owner, 7/23/2008

Nanostructured Material Electron Microscopy Images

Scanning Electron Microscopy
image taken by Dr. Suh

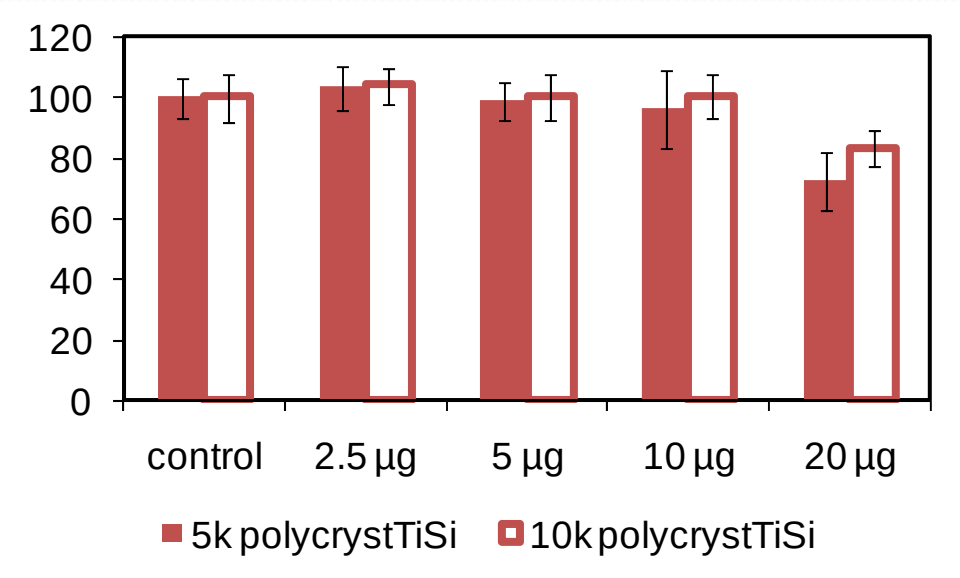
Nanostructured Material Electron Microscopy Images (cont.)



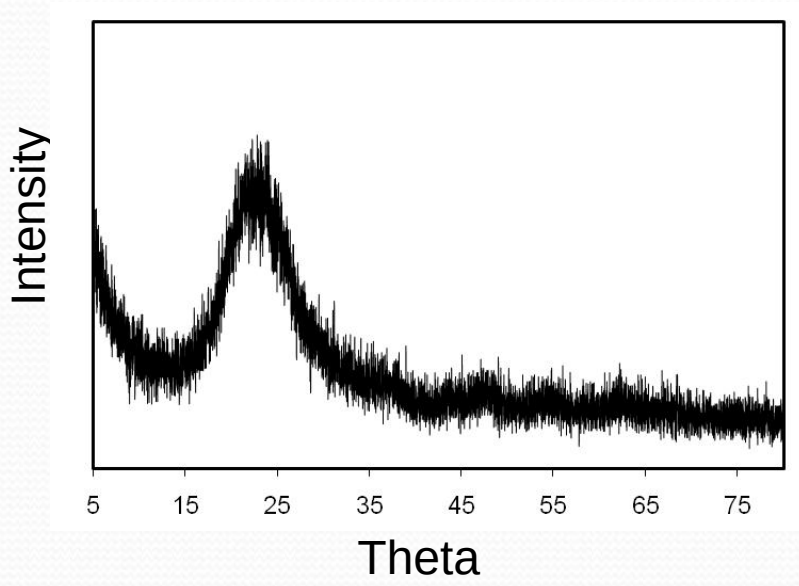
Scanning Electron Microscopy image taken by Dr. Suh

Cytotoxicity of Particles

Cytotoxicity data for BV2 cells
5k = 5,000 cells, 10k = 10,000 cells
Particle incubation time = 9 hours
WST-8 incubation time = 2.5 hours
polycrystTiSi = polycrystalline TiO_2 (not amorphous but getting small in size) on amorphous SiO_2

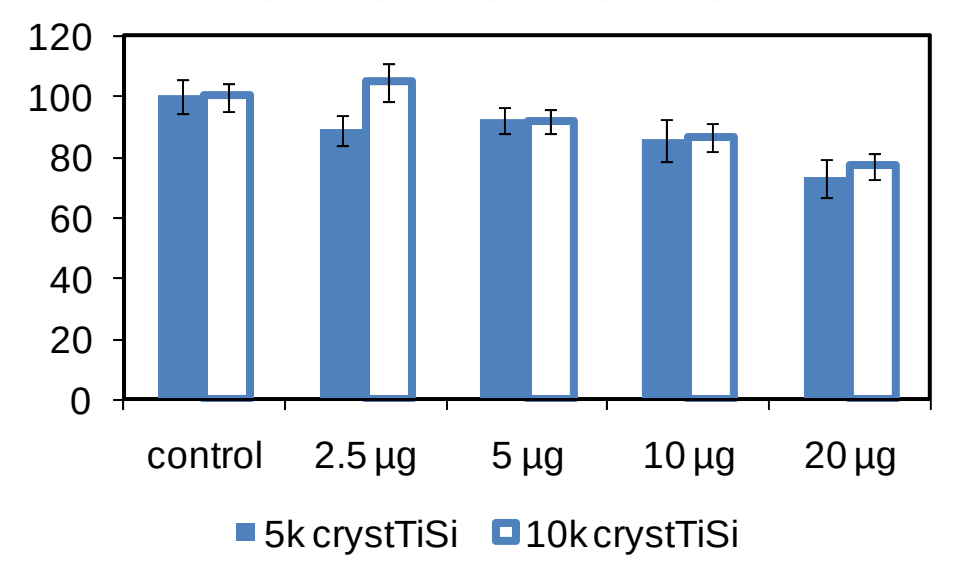


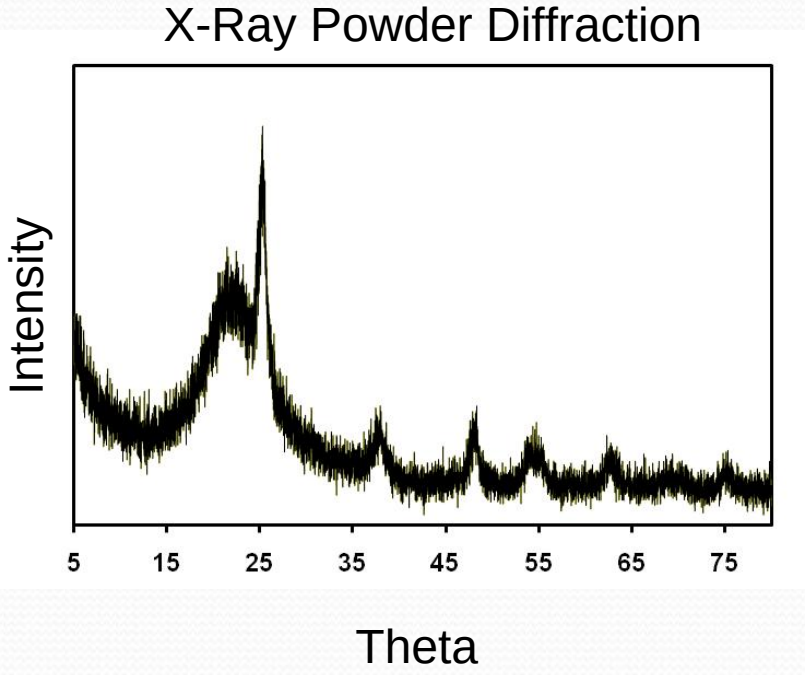
X-Ray Powder Diffraction



Cytotoxicity of Particles

Cytotoxicity data for BV2 cells
5k = 5,000 cells, 10k = 10,000 cells
Particle incubation time = 9 hours
WST-8 incubation time = 2.5 hours
crystTiSi = crystalline anatase TiO_2 on amorphous SiO_2





Conclusions

- Hands-on cell biology training
- Test particles were analyzed via WST-8
- Testing sub-50 nm TiO_2 Nanoparticles
- Polycrystalline TiO_2 toxic at high concentration
- Crystalline TiO_2 toxicity increased with concentration

Future Plans

- UV-VIS Spectroscopy for adsorption studies
- Additional cell biology experiments

Acknowledgments

Dr. Won Hyuk Suh

Prof. Galen D. Stucky

Mr. Jason Bedford

The Stucky group

INSET & CNSI

EPA (Environmental Protection Agency)

Otis Williams Postdoc Fellowship