Challenges in Conducting Hazard Assessment of NP

BIO1: Environmental Fate and Possible Effects of Nanoparticles
The 24th
Jyväskylä Summer School
Jyväskylän yliopisto

Nanoparticle Use

find Nano

AT&T 3:41 PM

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The Project on Emerging Nanotechnologies

at the Woodrow Wilson International Center for Scholars

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350TC Nano-Tex® Sheet Set by Studio

3XDRY® ESSEX SHIRT

45nm Processors

4Season OG Pants

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All Products | Detail

APO-HG binocular

Minox

PRODUCT WEBSITE

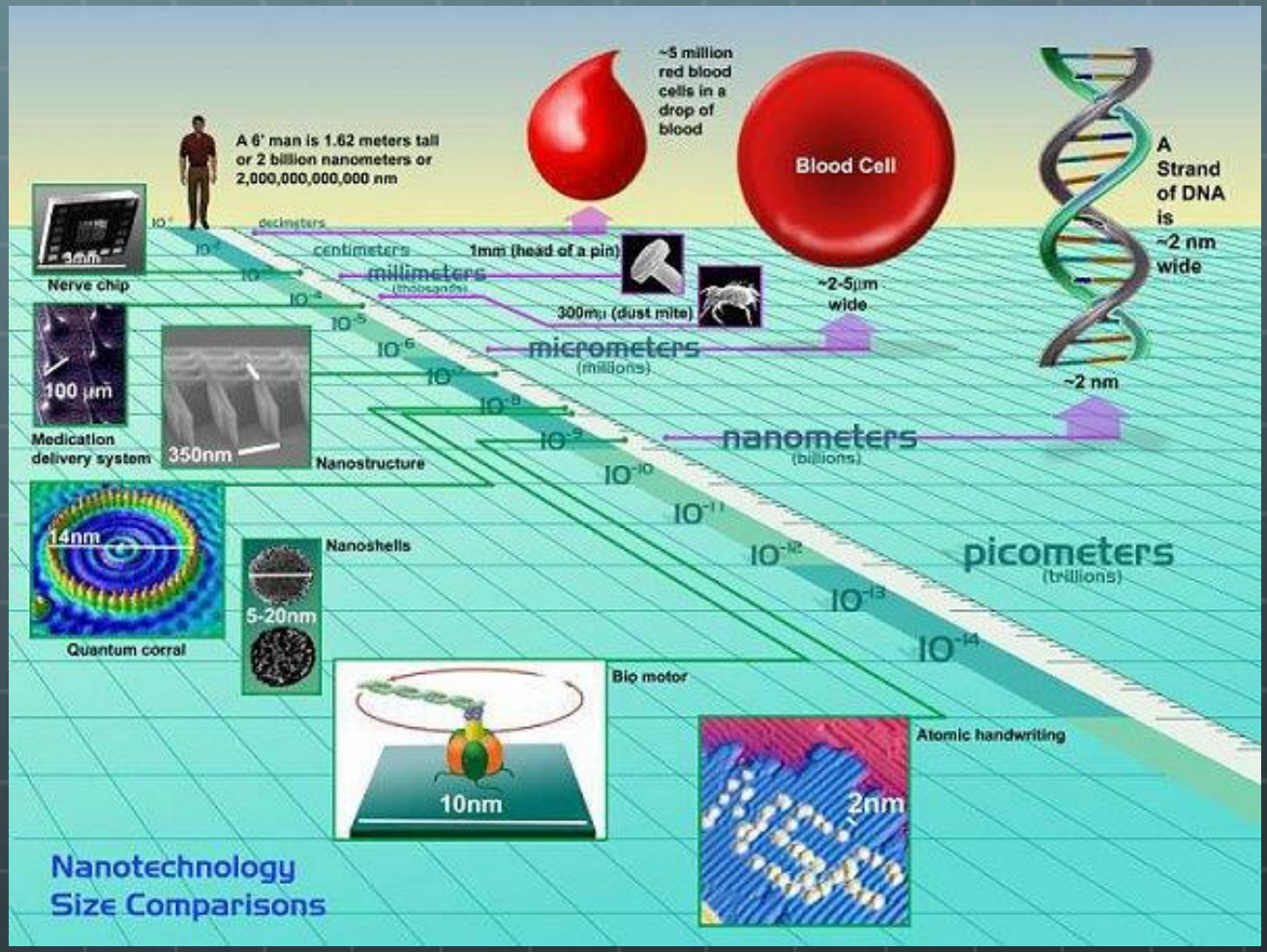
Date Added:
June 5, 2009

What They Say

SOURCE

"Pin-sharp optics with natural color rendition puts the new APO-HG binoculars from MINOX in a class of their own and first in the top league. The APO-HG 8.5x43 BR and the APO-HG 10x43 BR models impressively represent the latest technologies in binocular design. Featuring

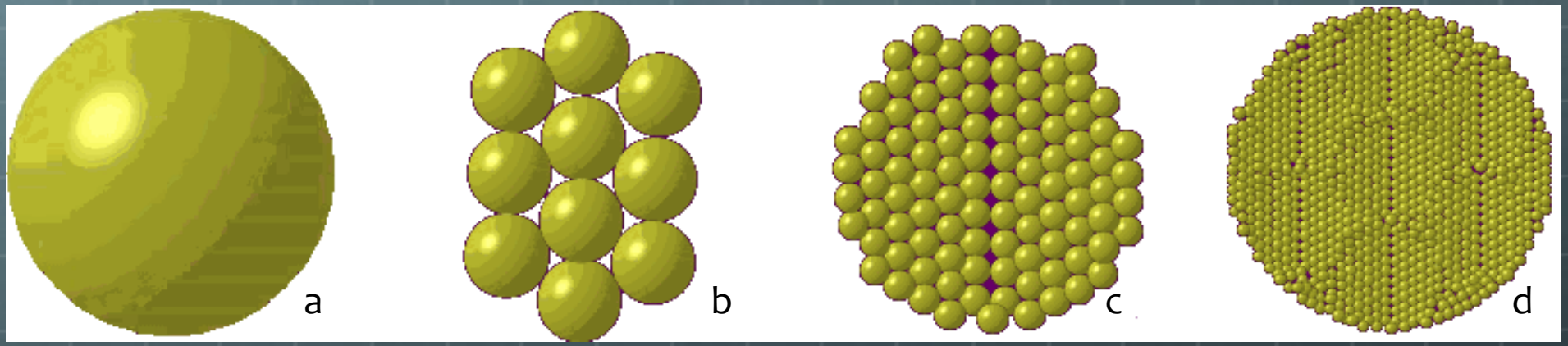
Nanoparticles are NOT solutes



How do you measure concentration?

- mg/l
- Particles/l
- Surface area
- other

Particle Properties



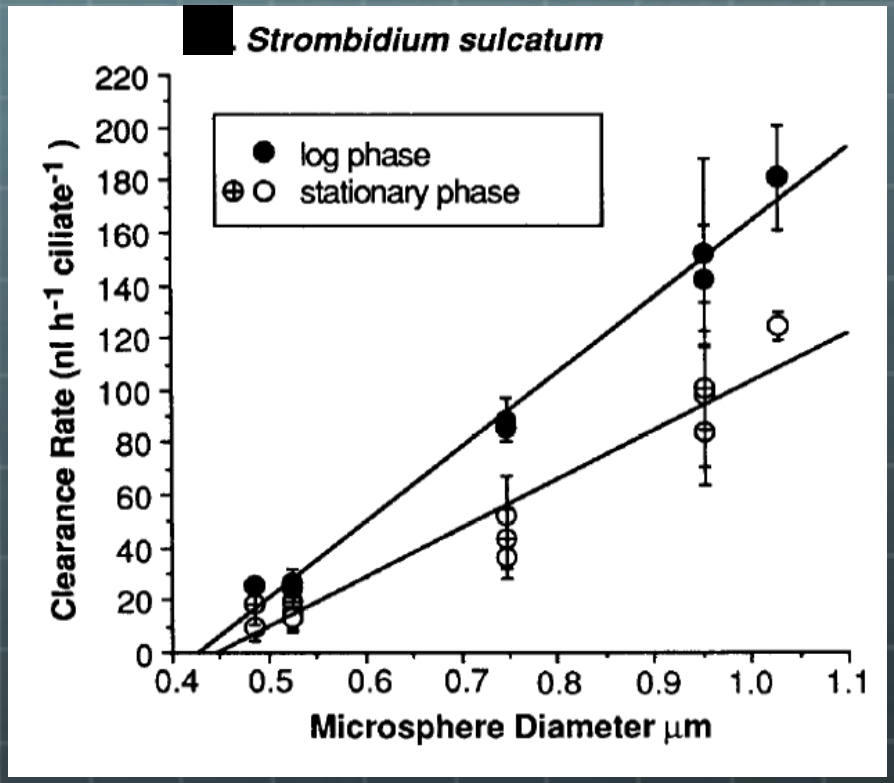
Gold Mass (µg)	1	1	1	1	1	1	1	1	1	1
Number of Particles	1[a]	10[b]	100[c]	1,000[d]	10,000	100,000	1,000,000	10,000,000	100,000,000	1,000,000,000
Particle Size (nm)	46,265.29	21,474.44	9,967.55	4,626.53	2,147.44	996.76	462.65	214.74	99.68	46.27
Particle Surface Area (nm^2)	6.72E9	1.45E10	3.12E10	6.72E10	1.45E11	3.12E11	6.72E11	1.45E12	3.12E12	6.72E12

Nanoparticle Behavior

Challenges

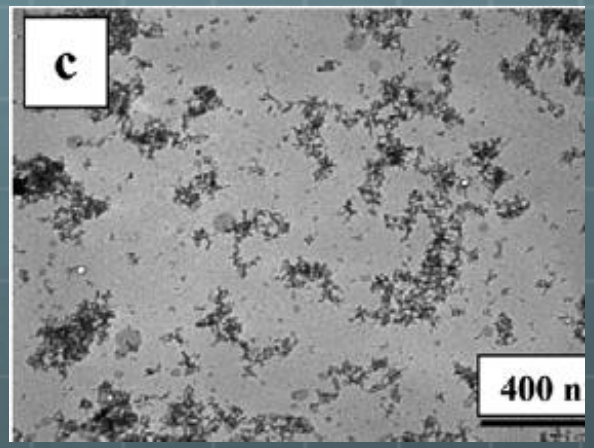
- Batch to batch variability
- Not truly soluble: colloidal suspension
- Quantification
  - Separating particles from ions and natural background concentrations (use of stable isotopes for Zn, Cu, different analysis techniques)

Nanoparticles in Aquatic Ecosystems

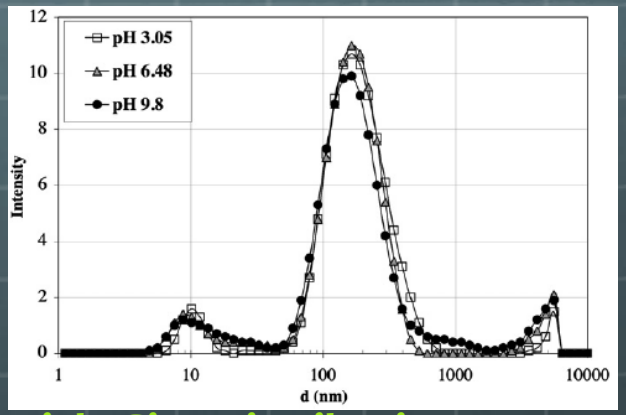


Nanoparticle size matters to filter-feeders
(Marine ciliate) Christaki et al 1998

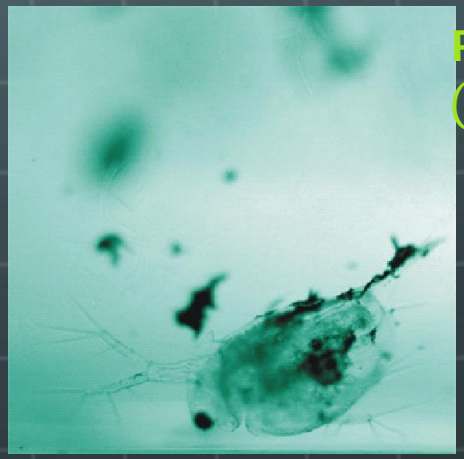
Nanoparticles in Aquatic Ecosystems



Particle Aggregation
(Fullerols) Brant et al 2007



Particle Size Distribution
(Fullerols) Brant et al 2007



Particle Stability
(SWNT) Roberts et al 2007

Nanoparticles in Aquatic Ecosystems

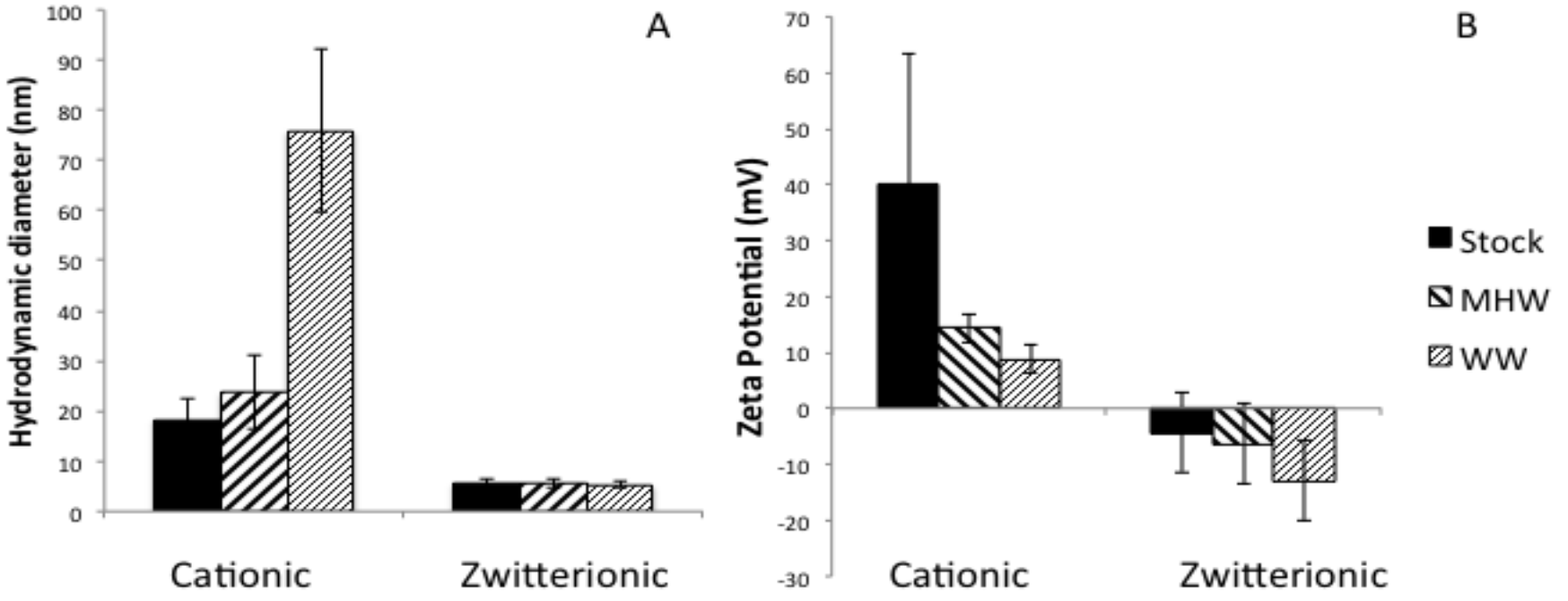


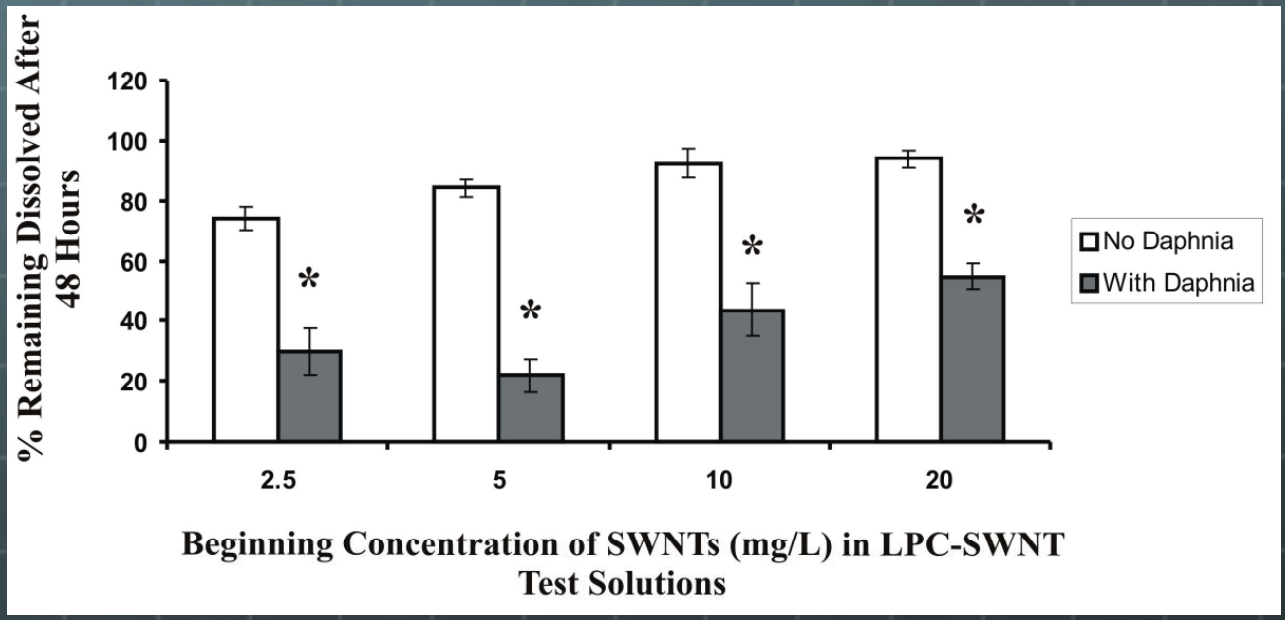
Figure 2. Hydrodynamic diameter (a) and zeta potential (b) for both nanoparticles in stock solution, moderately hard water and wastewater (after 1 hour). Each bar represents the average of three runs ± 1 standard deviation.

Aqueous conditions can also modify particle characteristics

Wray et al 2014, *In Prep*

Nanoparticles in Aquatic Ecosystems

Filter-feeders modify nanoparticle suspensions

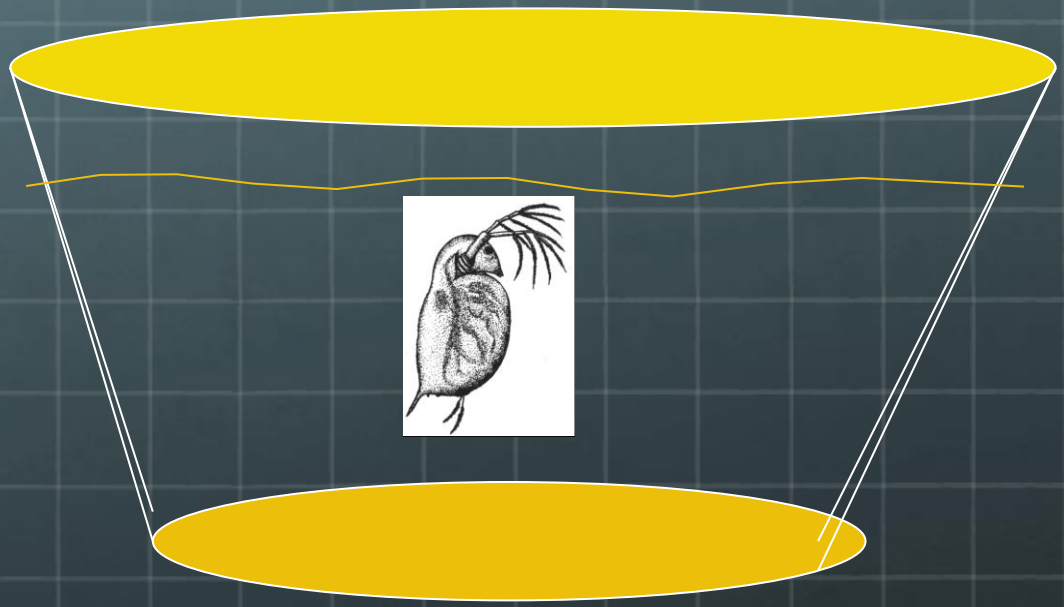


(SWNT) Roberts et al 2007

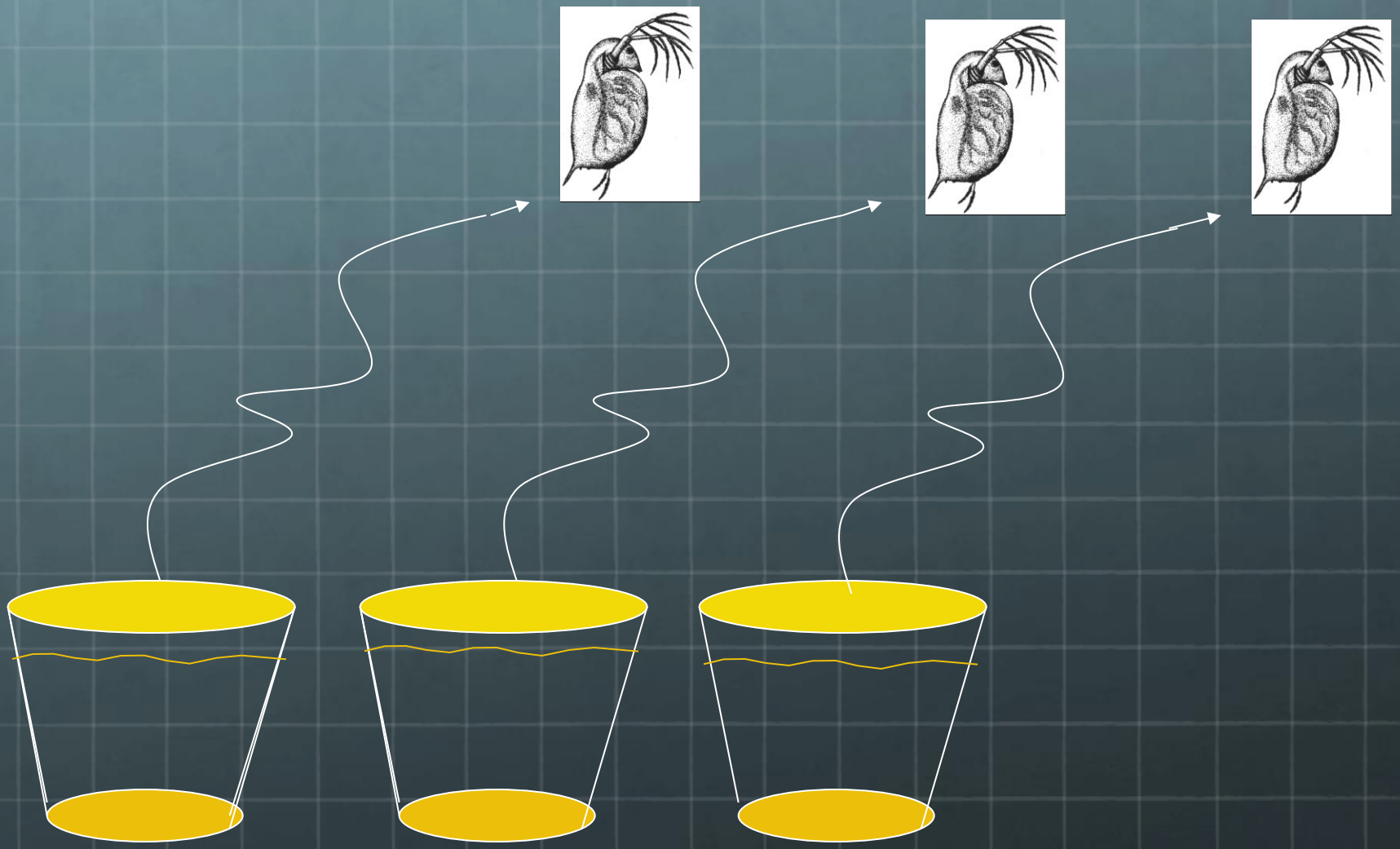
How do you test the effects of particles?

- Static bioassays
- Static renewal bioassays
- Flow-through bioassays

Static Bioassay



Static-Renewal Bioassay



Flow-through Bioassay

Current Environmental Research

- Research publications on environmental fate and effects increasing exponentially
- Initial research pointed out potential issues but also raised questions about research methods.
- Not enough to simply report how much was put into the test medium.
  - Size
  - Shape
  - Surface chemistry
  - Aggregation rate

Experimental Design

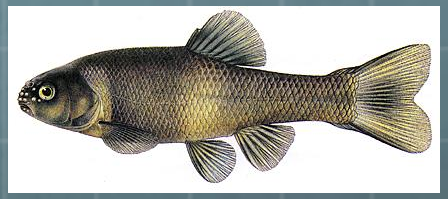
- Depends on the question
  - How toxic is the nanoparticle?
  - What is the mechanism of toxicity?
  - Can toxicity be attributed to the particle itself or is it a function of particle dissolution (particularly important for Ag, Cu and ZnO)?
  - What is the influence of size, shape, or surface chemistry on nanoparticle toxicity?
  - Does toxicity change based on the exposure media?
    - pH
    - Hardness
    - DOC
  - How does toxicity change between species?

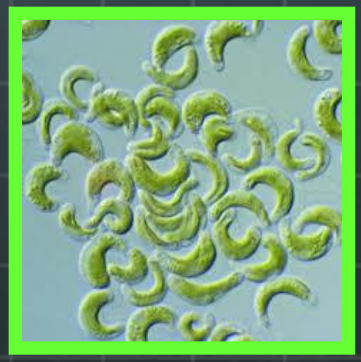
How toxic?

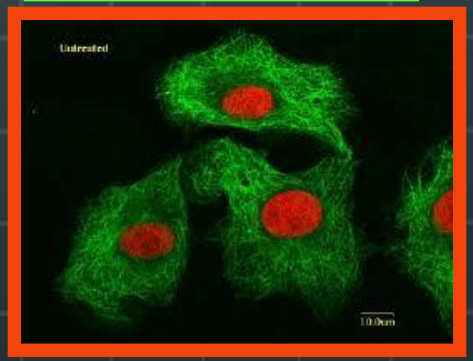
- Start with a standard bioassay procedure.
- Try to achieve constant exposure.
- Characterize particles during exposure
  - Do they aggregate, agglomerate (reversible), sediment, sorb to other surfaces, adsorb organic compounds, etc.

ENTOX Research

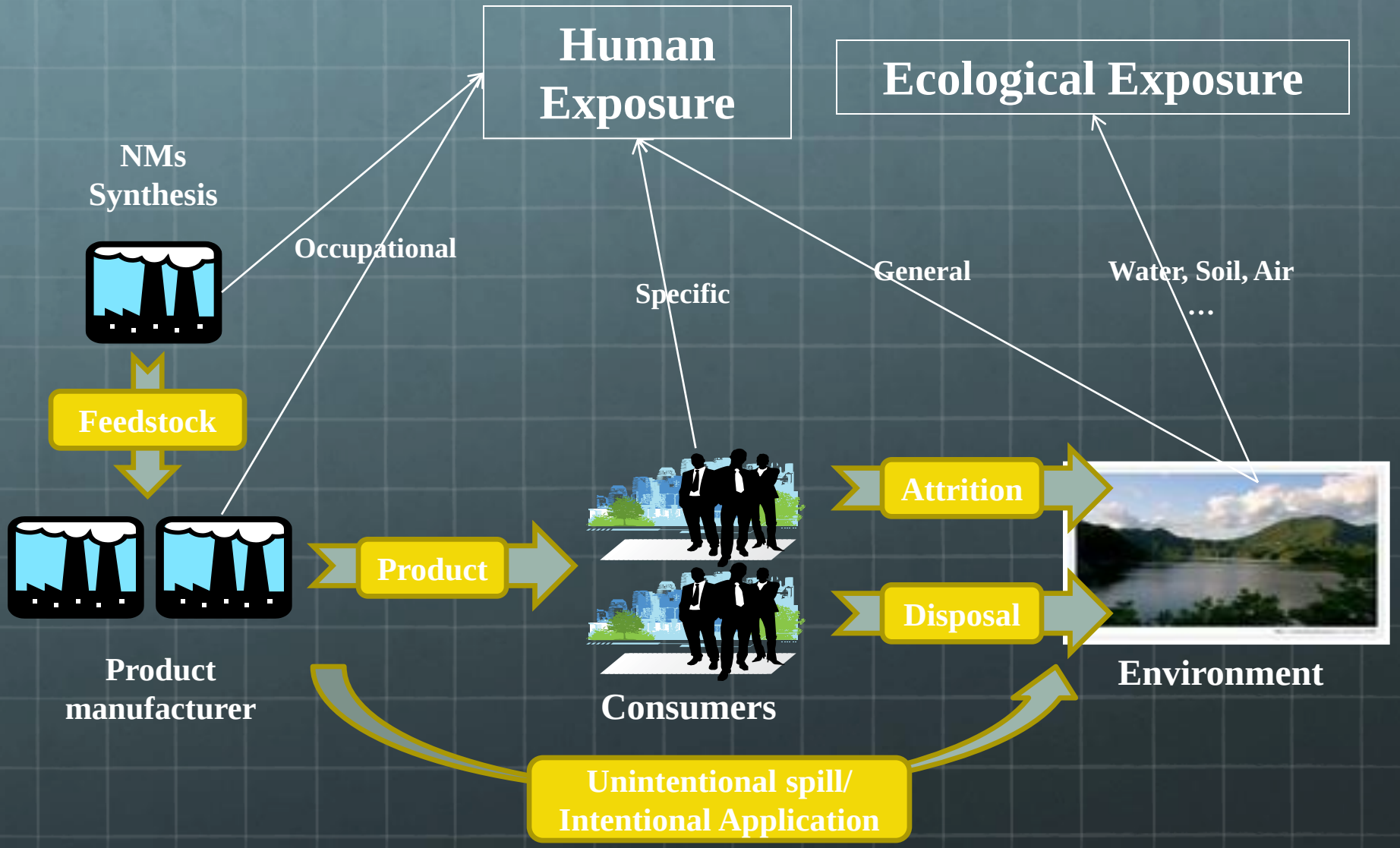
Complexity







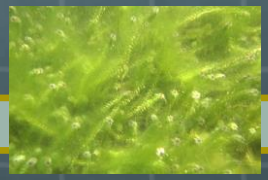
Release and Exposure of Nanoparticles



Why Aquatic Toxicology?

Human Exposure

General



Water, Soil, Air

…

Environment

Ecosystem Effects

Ecosystem Exposure

Product manufacturer/
Waste/treatment

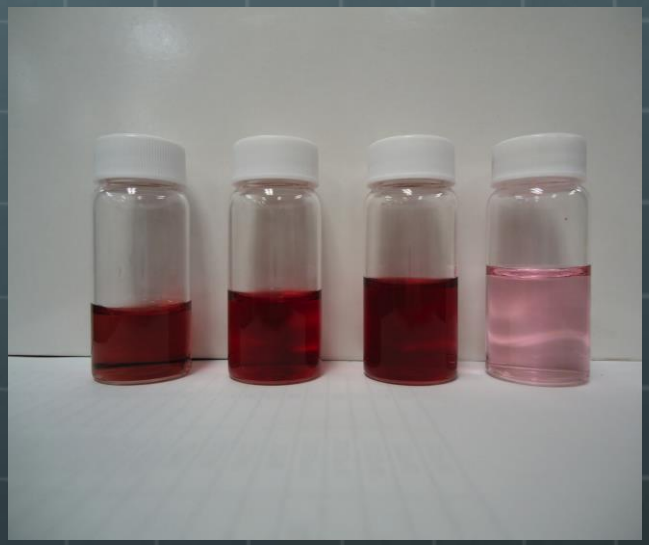
What is the Influence of Nanoparticle Characteristics?

- Choose nanomaterial
- Choose characteristics
  - Run a factorial design
  - e.g. 3 sizes, 3 shapes, 3 surface chemistries
  - 3 x 3 x 3 = 27 treatments
- Do characteristics change based on the exposure media?

What is the Influence of Particle Characteristics on the Toxicity of Gold Nanoparticles

Particle Core	Shape	Size	Surface Charge	# treatments/ Bioassay
Gold	Sphere	4, 10, 50 nm diameter	Amine (protonated) (cationic) Biotin (nonionic) Carboxylate (anionic)	3 3 3
	Cube	50, 75 nm	Amine (protonated) (cationic) Biotin (nonionic) Carboxylate (anionic)	2 2 2
	Rod	20 by 100 nm 20 by 400 nm	Amine (protonated) (cationic) Biotin (nonionic) Carboxylate (anionic)	2 2 2

Stock Solutions of In-House AuNPs					
Shape	Sphere	Sphere	Sphere	Rod	Rod
Particle Size (nm)	5.67 ± 1.28	21.25 ± 2.5	30.64 ± 6.00	W: 17.82 ± 2.03 L: 58.08 ± 5.31	W: 17.82 ± 2.03 L: 58.08 ± 5.31
Surface Chemistry	Citrate	Citrate	Citrate	Poly(acrylic acid)	Poly(allylamine hydrochloride)
Zeta Potential (mV)	-39.8 ± 9.94	-35.7 ± 19.5	-38.9 ± 16.4	-20.7 ± 9.33	+38.8 ± 17.5

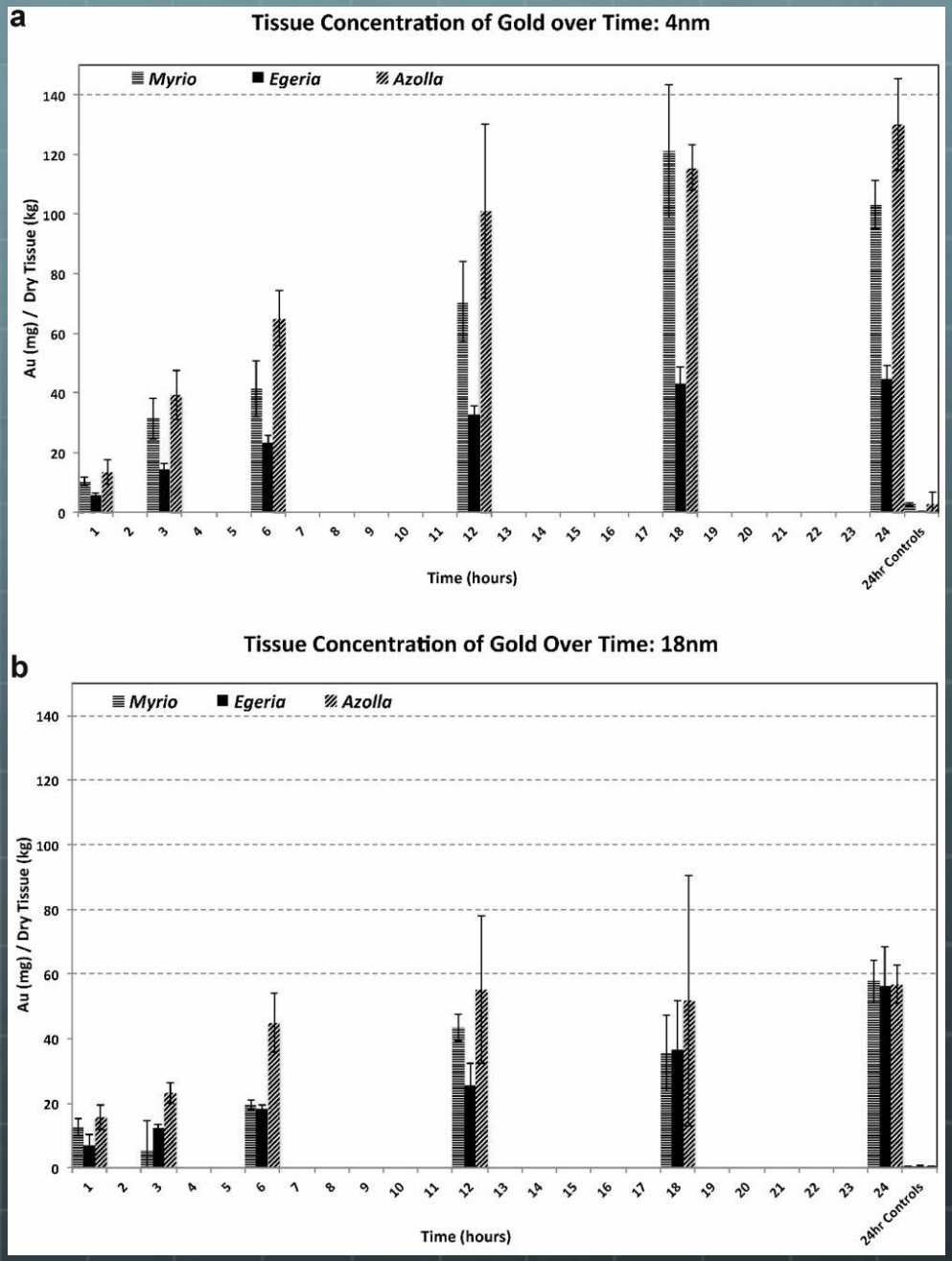


Size (nm)	Shape	Surface Ligand	k_{uw} (L g_{org}^{-1} d^{-1}) [a]	k_e (d^{-1})
4	Sphere	Citrate	5.139 ± 0.388	2.929 ± 0.140
20	Sphere	Citrate	2.772 ± 0.247	1.840 ± 0.190
30	Sphere	Citrate	2.679 ± 0.120	1.119 ± 0.213
18 x 58	Rod	Poly(acrylic acid)	1.548 ± 0.038	2.025 ± 0.287
18 x 58	Rod	Poly(allylamine hydrochloride)	L: 4.632 ± 0.830 H: 92.494 ± 6.504	2.746 ± 0.303

Individual rate constants for each particle configuration

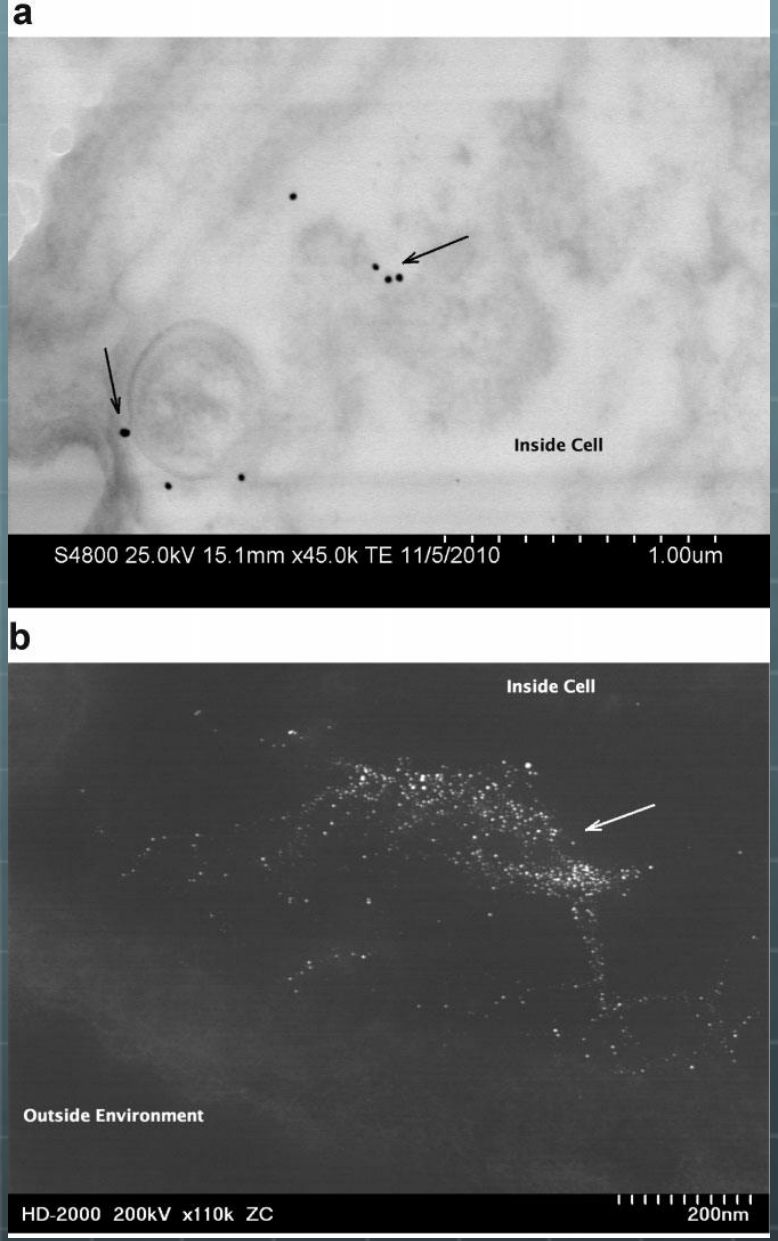
Model predictions for rate constants and BCF using full data set and multiple linear regression analysis

Diameter (nm)	Surface Charge	k_{uw} (L g_{org}^{-1} d^{-1})	k_e (d^{-1})	BCF
4	Anionic	5.14	2.93	1750[a]
20	Anionic	2.81	1.93	1460[a]
30	Anionic	2.81	1.12	2510[a]
4	Cationic	L: 5.14 H: 94.83	3.74	L: 1370 H: 25400
20	Cationic	L: 2.80 H: 92.02	2.75	L: 1020 H: 33500
30	Cationic	L: 2.80 H: 92.02	1.93	L: 1450 H: 47700

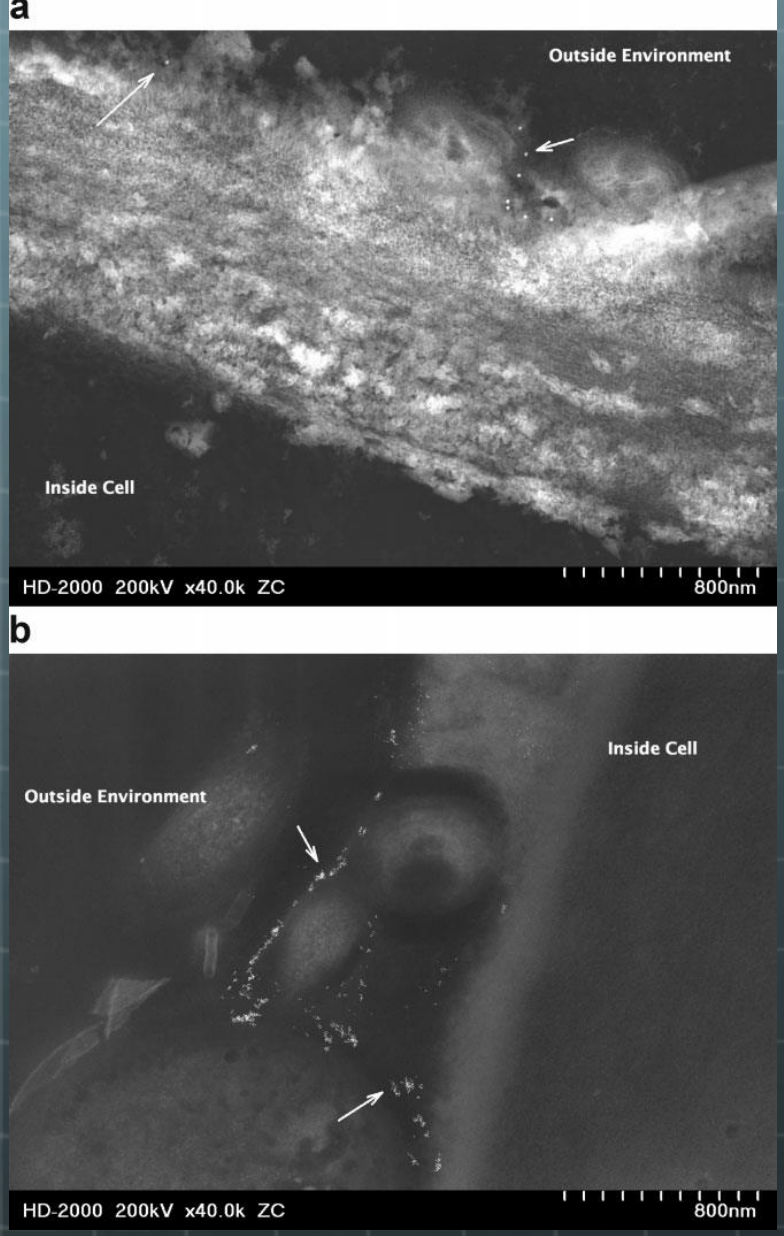


Species Differences

- Aquatic macrophytes exposed to citrate coated gold nanoparticles with 4 and 18 nm diameter
- Accumulation is both size and species dependent



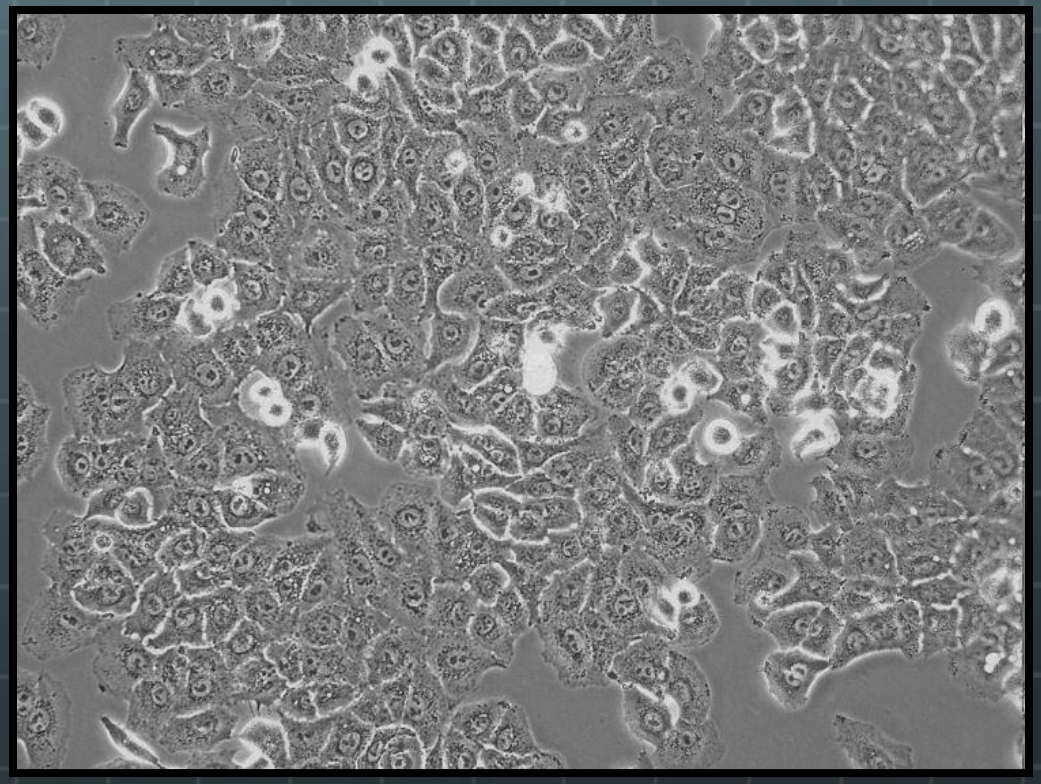
A. caroliniana exposed to 18 (a) and 4 nm (b) gold NPs



E. densa exposed to 18 (a) and 4 nm (b) gold NPs

Influence of Expsoure Media

- A549 Cells
  - human carcinomic alveolar epithelial cells
  - Squamous Cells extracted in 1972



Cell Culture Methods

- Dulbecco's modified Eagle's Media
  - 10 Fetal Bovine Serum (FBS)
  - 2mM Glutamine
  - 1% Non-essential AA's
  - 2% Penicillin/Streptomycin
- 75cm^2 TC-Treated Flask
- 5% CO_2 at 37 °C

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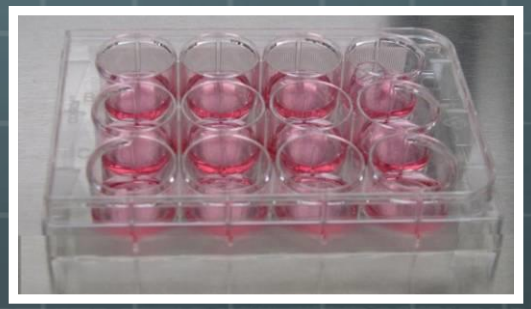
Experimental Methods

- 12-well TC-Treated Plates
  - 10^5 cells per well
- 24hr for Attachment
- Fresh Media + Particles
- n=3
- Rinsed with PBS (x3)
- 40% aqua regia
- Analyzed ICP-MS



Primary Objective

To develop a rapid, high volume bioassay to facilitate investigations of a large array of particle modifications

1. Exposure Media

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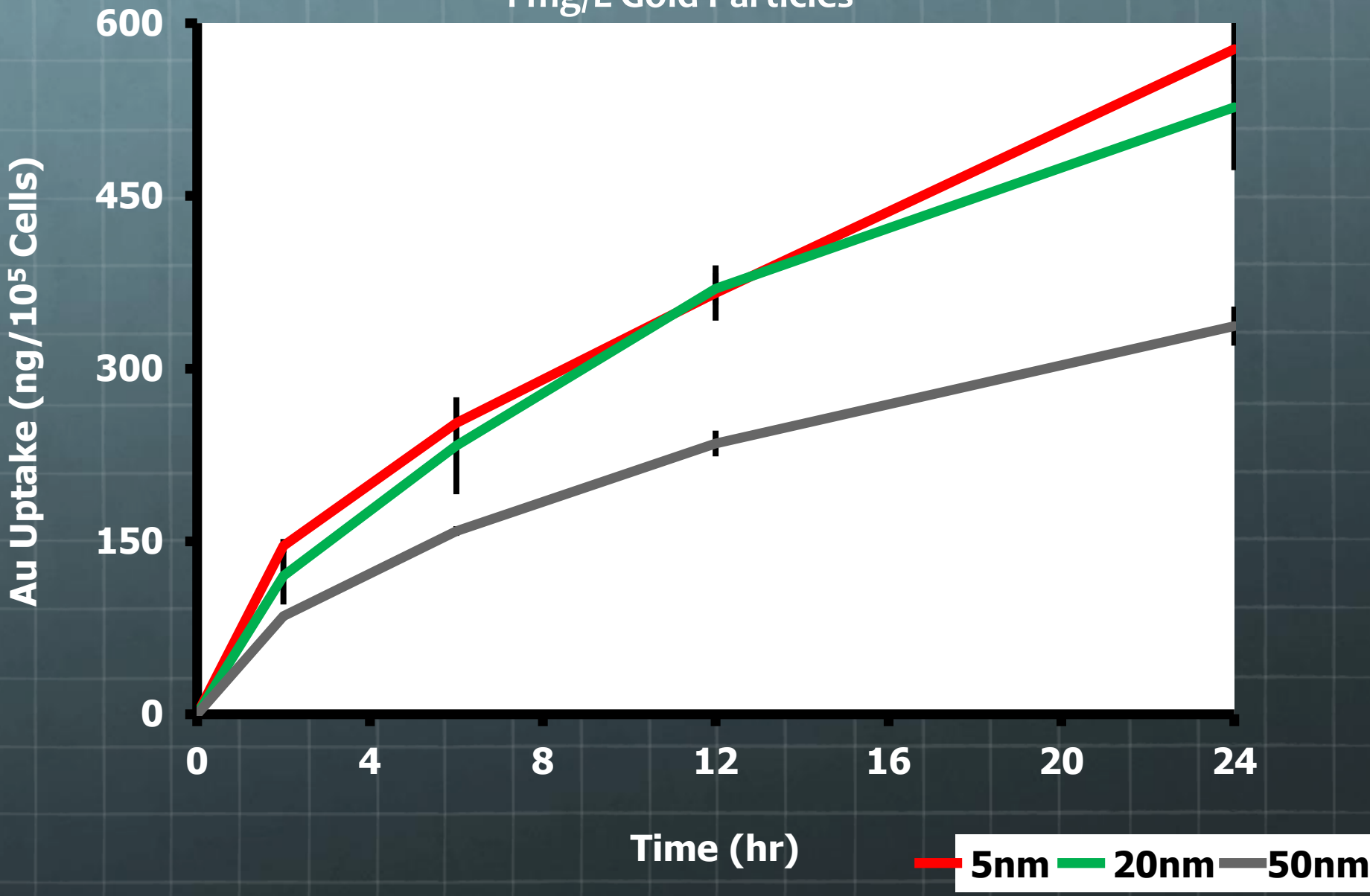
To develop a rapid, high volume bioassay to facilitate investigations of a large array of particle modifications

1. Exposure Media
   - 5% FBS
   - 10% FBS
   - 20% FBS

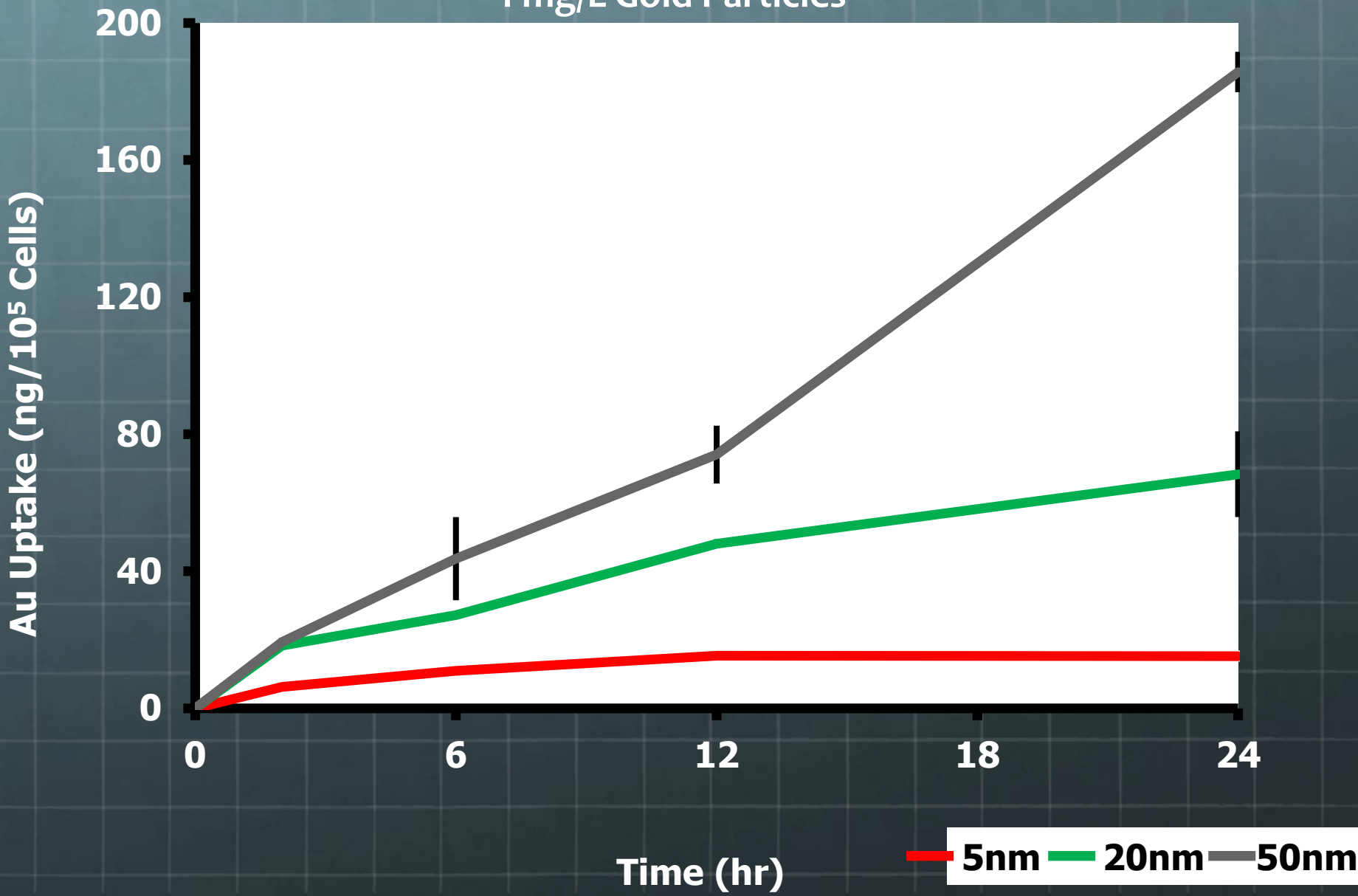
5% FBS

1 mg/L Gold Particles



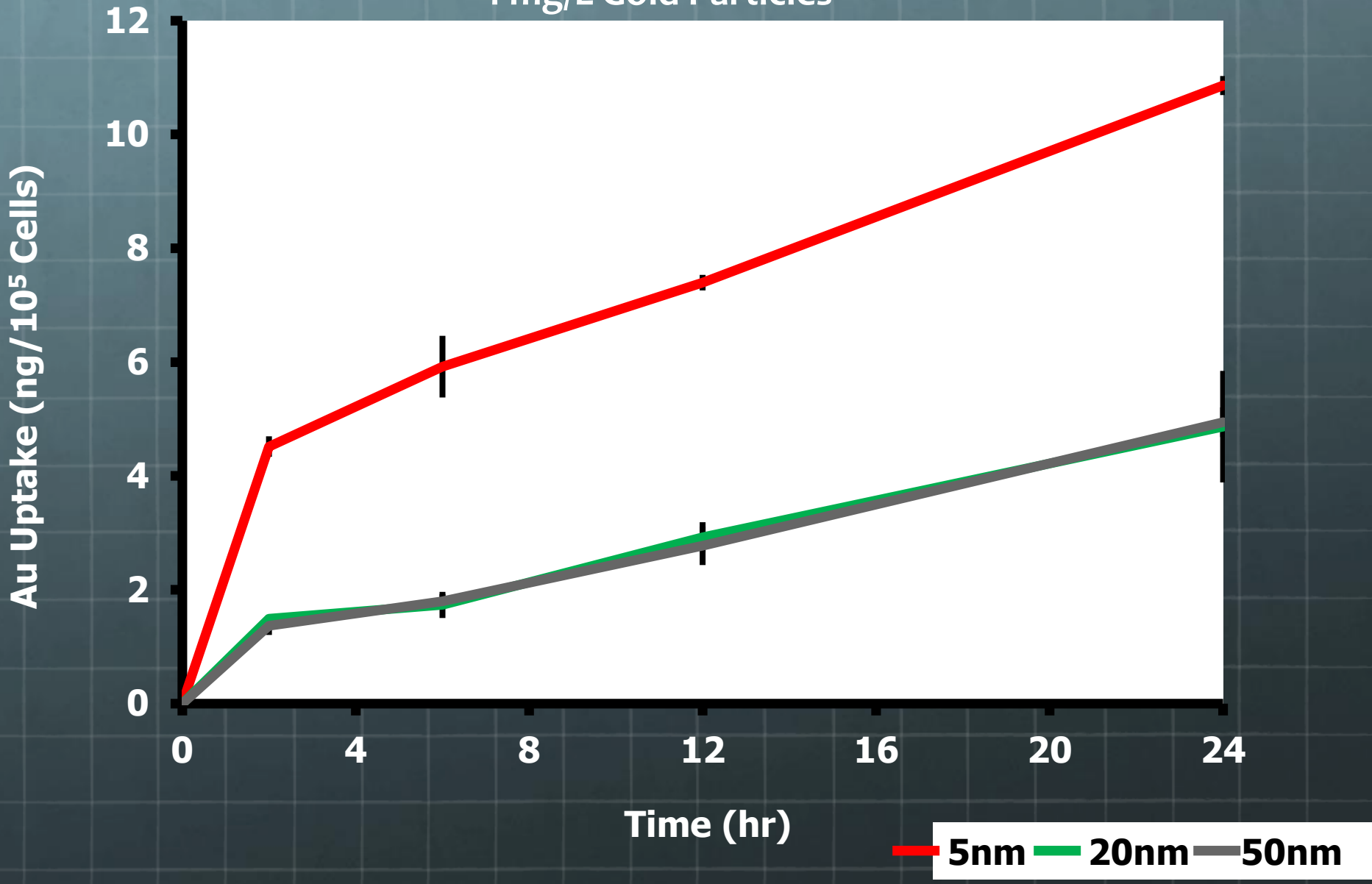
10% FBS

1 mg/L Gold Particles

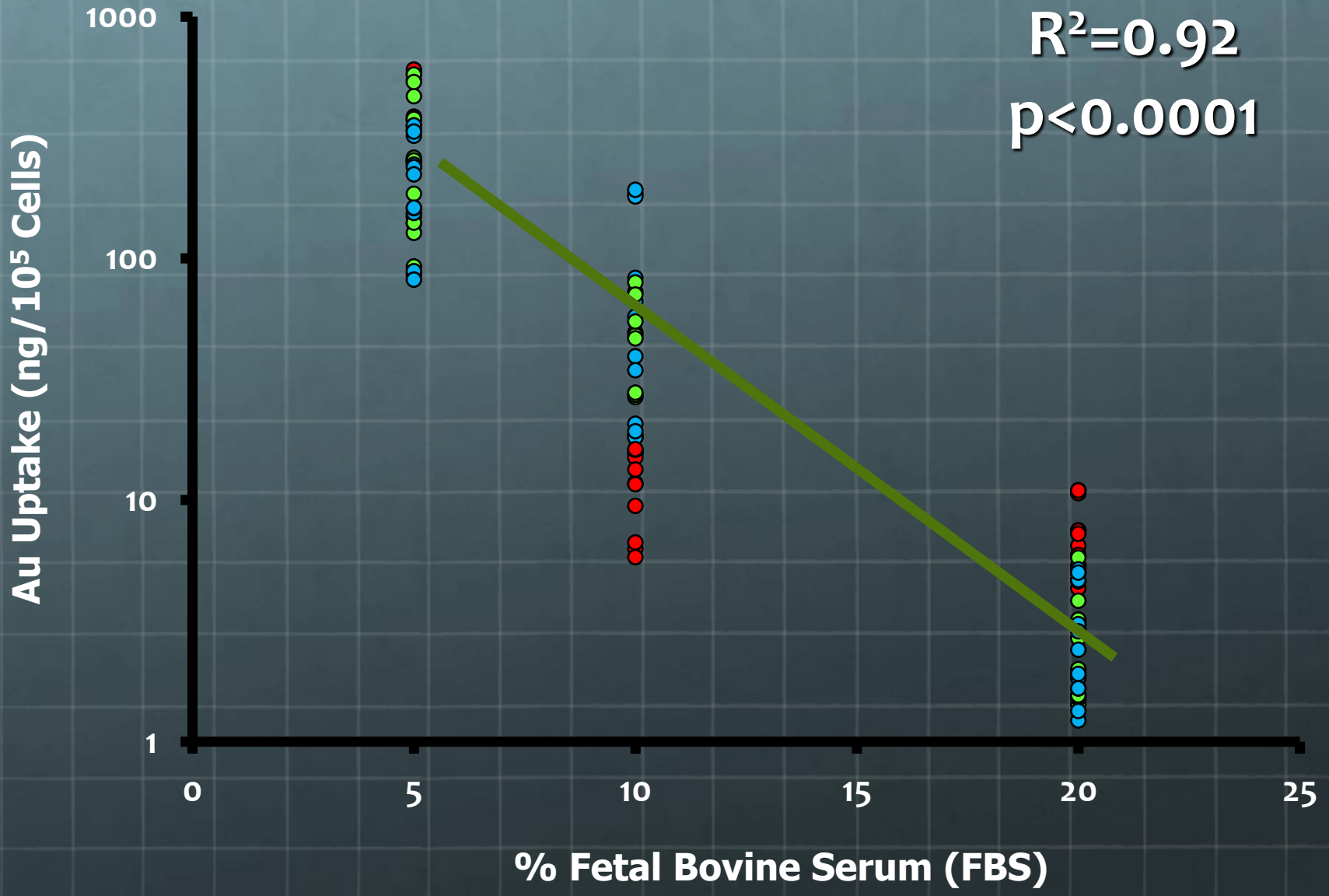


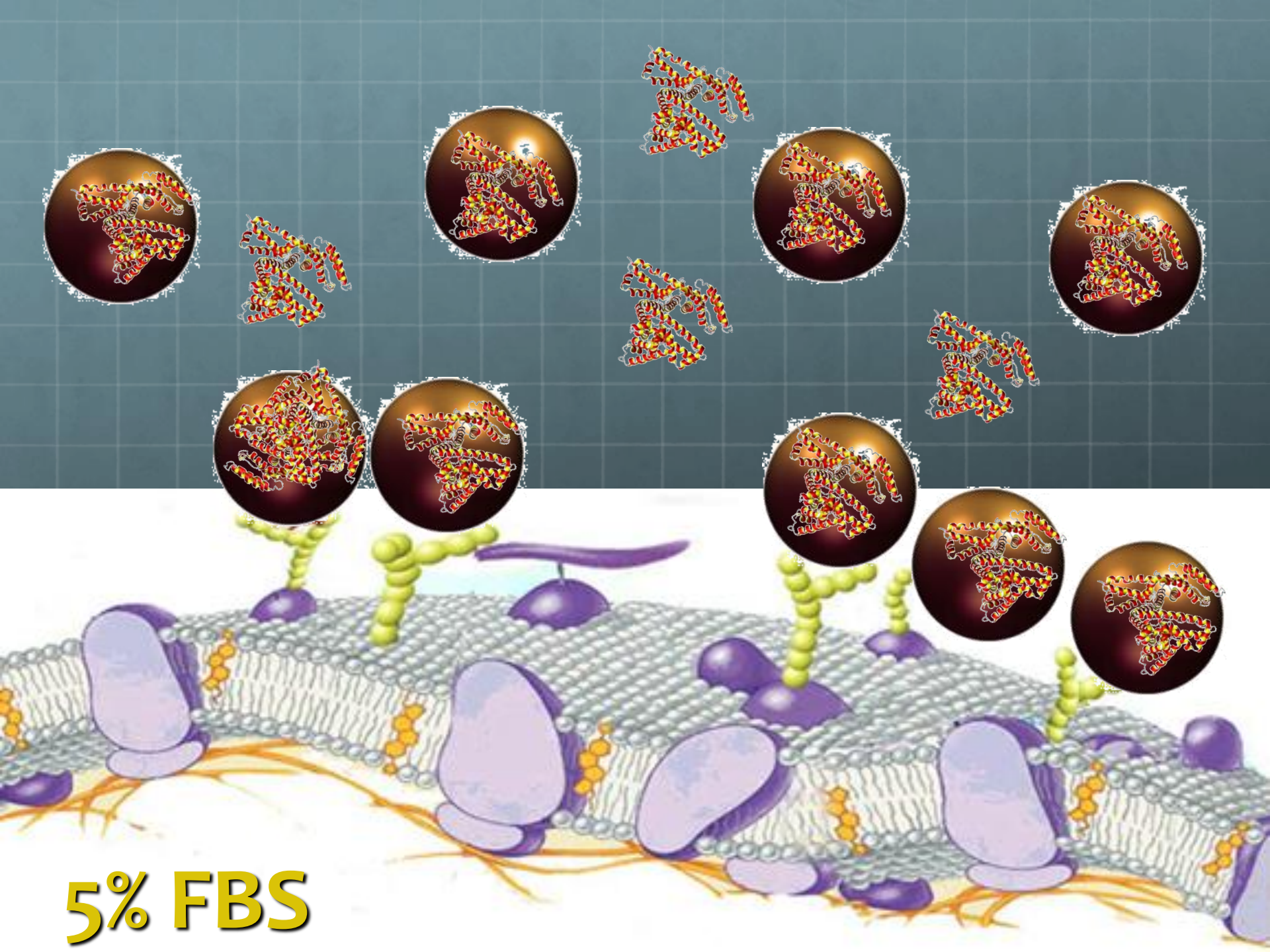
20% FBS

1 mg/L Gold Particles



Regression


R^2=0.92
p<0.0001
Au Uptake (ng/10^5 Cells)
1000
100
10
1
0
5
10
15
20
25
% Fetal Bovine Serum (FBS)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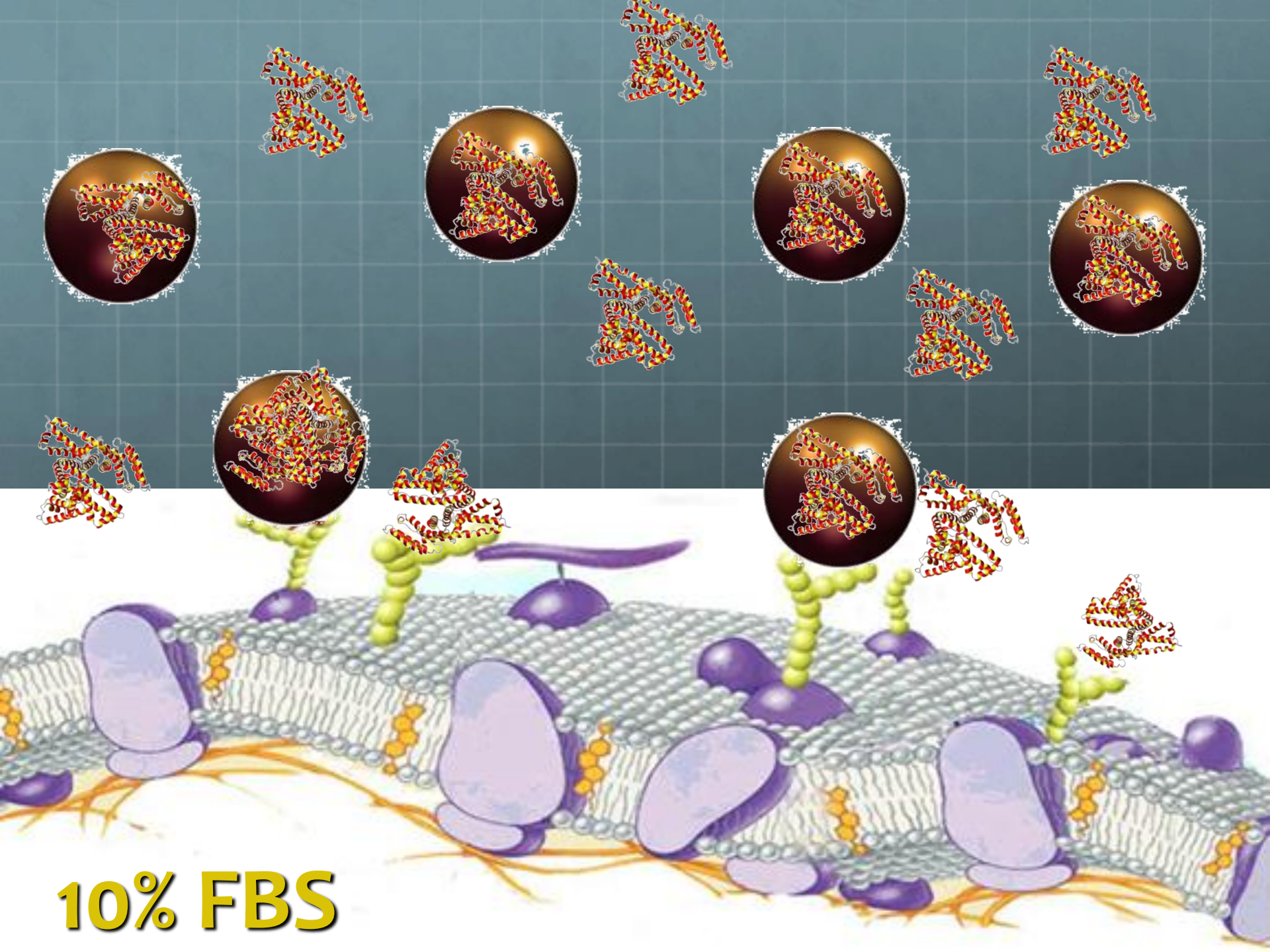
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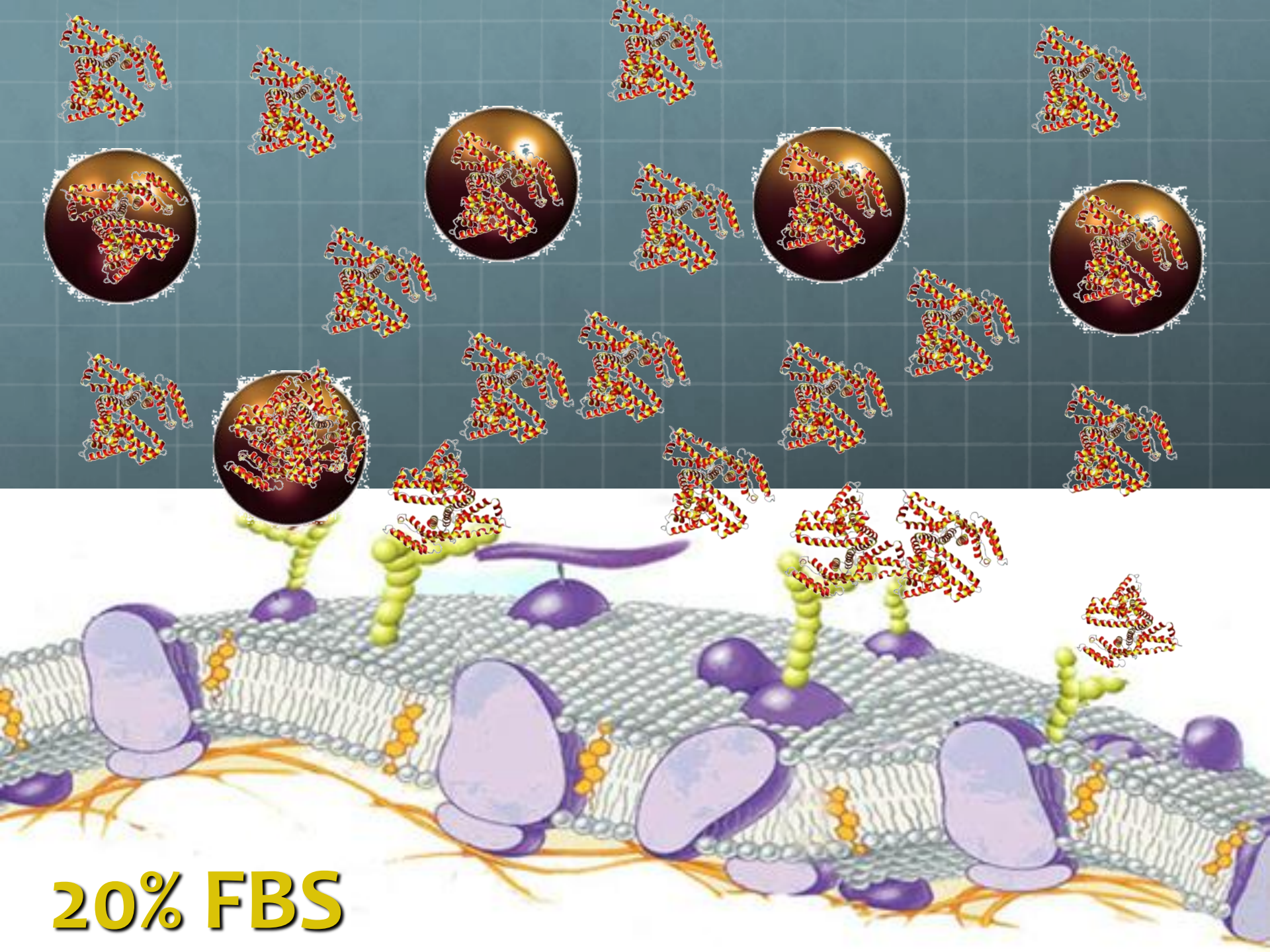
20% FBS