Nanomanufacturing Summit 2010
Lowell
June 22-24, 2010

Engineered Nanomaterials: Linking Physicochemical Properties with Biology

by

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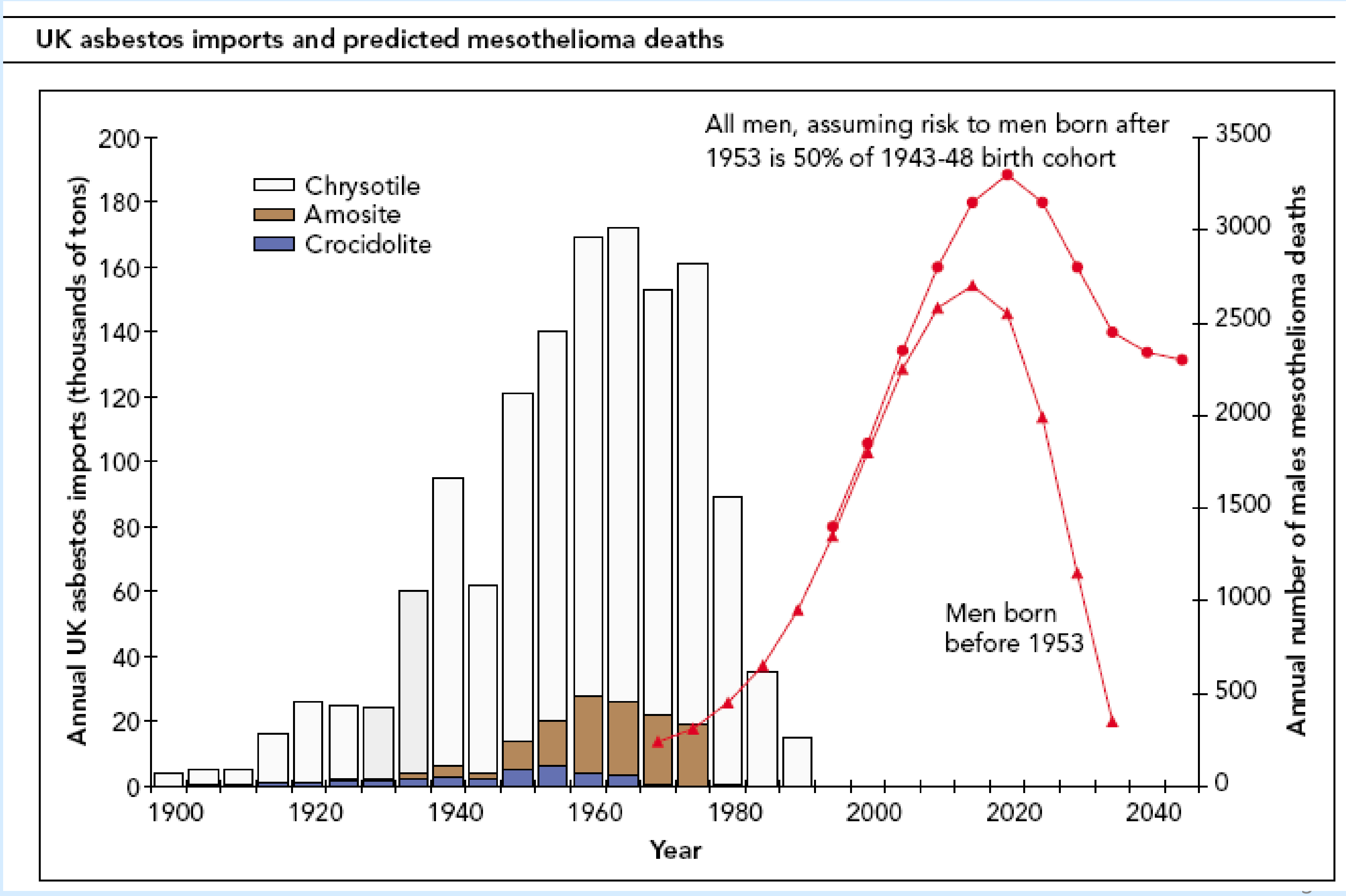
Outline

- **Over view of the safety concern of nanomaterials**
- **Challenge of knowing toxicity of nanomaterials**
- **BOD/ROS, nano exposure and adverse health effect**
- **Developing a screening test to predict toxicity of nanomaterials**
- **Linking Physicochemical Properties with Biology**

The Lesson from Asbestos



Unlearned Lessons from the Past

- Hansen SF, Maynard A, Baun A, Tickner JA. 2008. Late lessons from early warnings for nanotechnology. Nat Nanotechnol 3(8): 444-447.

"We are in danger of repeating old, potentially costly, mistakes."

Why?

Complexity of Nanomaterials

&

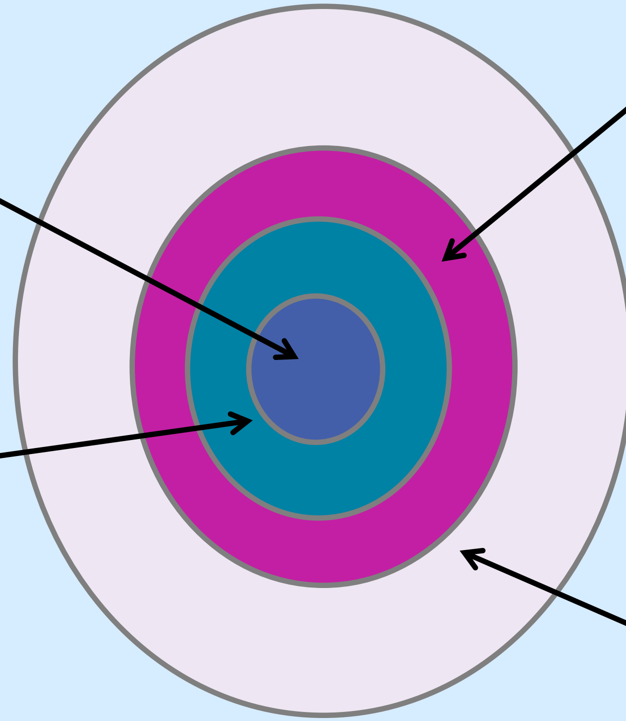
Uncertainty of Nanoparticle-Biomolecule Interaction

***A.* Basic Categories**

e.g. carbon base materials, metal oxides, elemental metals, Quantum dots, complex compounds, organic polymers, etc.

***C.* Surface Modification**

e.g. surface functionalization, coating, etc.



***B.* Physical Characteristics e.g. morphology, diameter, length, aspect ratio*,* crystallinity, etc.**

***D.* Formation of Secondary Structure by Agglomeration**

e.g. morphology, surface charge, hydrophobicity, surface reactivity

Increasing number of possibilities for different ENM's

Challenge of Knowing Toxicity of Nanomaterials

Physicochemical properties of nanomaterials
&
The interactions between these properties

↓

Interaction with biomolecules & cells
Distribution
Degradation /Accumulation
Toxicity/Adverse Health Effects

Huge Uncertainty

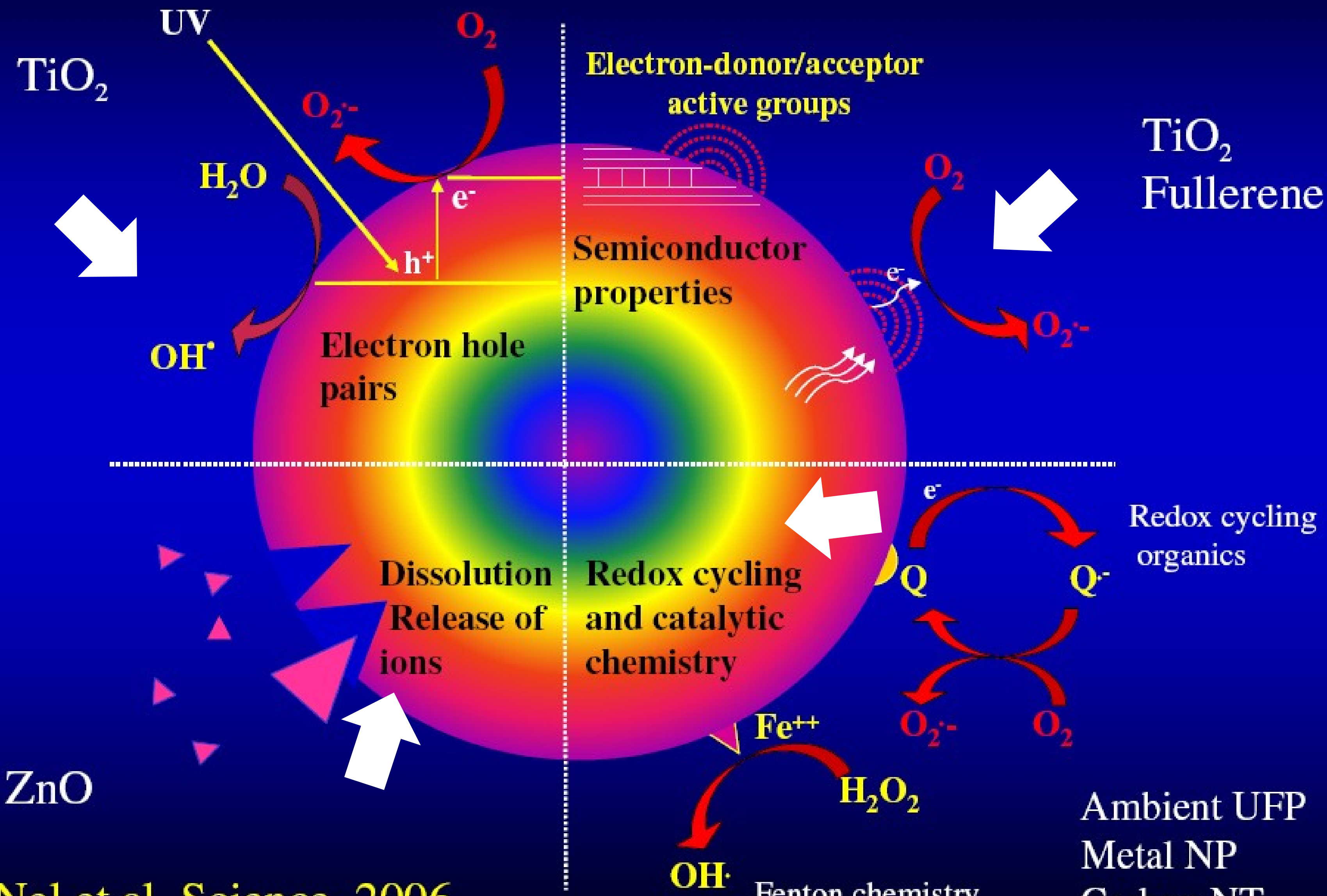
Challenge of Knowing Toxicity of Nanomaterials

- The link between PCs and toxicity remains poorly understood
- Robust screening approaches are still lacking
- What could be a key metric for screening test?
- How to quantify the key metric and estimate the potential toxicity?

The Possible Mechanisms of Nanotoxicity

- **Oxidative stress**
- **Catalytic Metal in ENMs** - catalyze reactive oxygen species generation
  toxic metal itself

Examples of Particle-mediated Oxygen Radical production



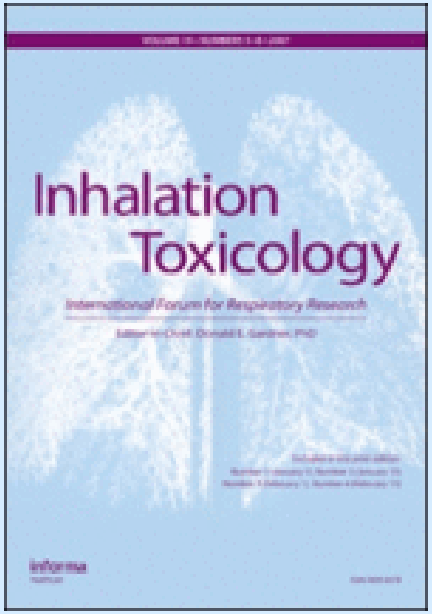
Nel et al. Science. 2006

The Possible Mechanisms of Nanotoxicity

- Oxidative stress
- Catalytic Metal in ENMs - catalyze reactive oxygen species generation
  toxic metal itself
- Membrane disruption – relate to oxidative stress & adsorption
- Essential nutrient or functional biomolecule depletion
- Structure alteration of functional biomolecules
- Others; immune toxicity

Criteria of a Toxicity Screening Test

- **Must be sensitive to a large number of physicochemical properties of diverse classes of ENMs that may elicit adverse effects in biological systems.**
- **Must be highly predictive of potential toxicity of multiple mechanisms.**
- **Must be relatively simple, sensitive, specific, robust, precise, low cost, exhibit low susceptibility to interferences and possess high throughput capability.**
- **Must be easily standardized to a highly recognizable endpoint.**



Inhalation Toxicology

International Forum for Respiratory Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713657711

Evaluating the Toxicity of Airborne Particulate Matter and Nanoparticles by Measuring [redacted] Potential - A Workshop Report and Consensus Statement

Jon G. Ayres [a]; Paul Borm [b]; Flemming R. Cassee [c]; Vincent Castranova [d]; Ken Donaldson [e]; Andy Ghio [f]; Roy M. Harrison [g]; Robert Hider [h]; Frank Kelly [i]; Ingeborg M. Kooter [j]; Francelyne Marano [k]; Robert L. Maynard [l]; Ian Mudway [m]; Andre Nel [n]; Constantinos Sioutas [o]; Steve Smith [p]; Armelle Baeza-Squiban [k]; Art Cho [n]; Sean Duggan [q]; John Froines [n]
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"Toxicity Screening tests for new nanomaterials products are urgently needed. Whilst recognizing that oxidative stress potential may not be predictive of all possible adverse outcomes, tests based upon oxidative potential maybe an invaluable tool for initial screening and classification of the relative biohazard of such materials."

The human study on association of particulate matter and diseases

Title	Journal	Reference
Associations of long- and short-term air pollution exposure with markers of inflammation and coagulation in a population sample	Occup. Environ. Med	Panasevich et al. 2009
Ambient Particulate Pollutants in the Ultrafine Range Promote Early Atherosclerosis and Systemic Oxidative Stress	Circ. Res	Araujo et al. 2008
Effects of air pollution on the incidence of myocardial infarction	Heart	Bhaskaran et al. 2009
Long-Term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women	N. Engl. J. Med	Miller et al.2007
Cardiovascular Mortality and Long-Term Exposure to Particulate Air Pollution	Circulation	Pope et al. 2004
Long-term exposure to traffic-related air pollution and mortality in Shizuoka, Japan	Occup. Environ. Med	Yorifuji et al.2010
Fine-Particulate Air Pollution and Life Expectancy in the United States	N. Engl. J. Med	Pope et al. 2009

Oxidative Damage or ROS Generation Could Be Used as a Metric for Nanotoxicity Screening

2. How to quantify oxidative stress or ROS generation ?

Assay Methods to Determine Reactive Oxygen Spices Generation

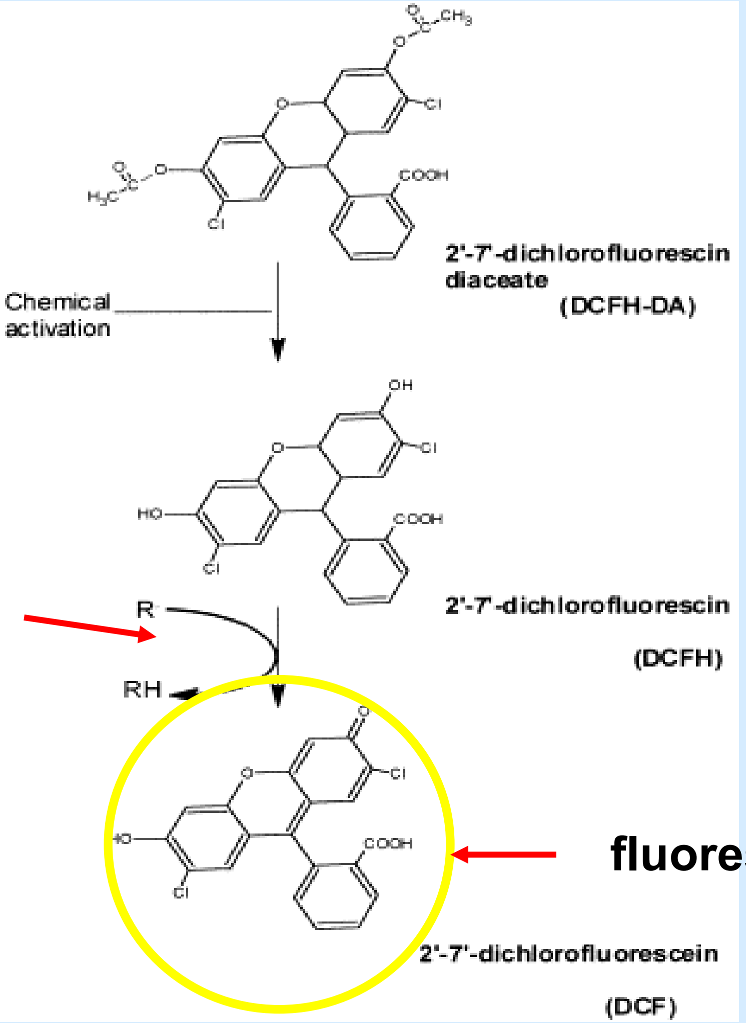
Assay	Target ROS	Advantages	Disadvantages	used in nano study
DCDHF	ROS	Can be applied intra- and extra-cellularly	Autocatalytic degradation, no information about ROS	√
ESR/EPR	Free radicals	Quantitative, structural information	in virto only/proficiency required	√
Antioxidants Inhibition				
FRAS	any type of ROS	Can be applied extra-cellularly	Little information about radical species	√
DTT consumption	any type of ROS	Can be applied extra-cellularly		√
Vitamin C yellowing	any type of ROS	Can be applied extra-cellularly		√
Chemiluminescence (salicylate catalyst)	ROS, •OH and ONOO−	Quantitative	Limited to •OH and ONOO-	

Assay methods to determinate reactive oxygen spices generation
Abbreviation: ROS- reactive oxygen spices, DCHF -2’,7’-dichlorofluores-cein, ESR-electron spin resonance, EPR-electron paramagnetic resonance, FRAS- ferric reducing ability of serum, DTT- The dithiothreitol assay,

DCFH vs. FRAS: Comparison

DCFH Method

NaOH



ENMs, H_2O_2, etc.

fluorescent compound

FRAS - Ferric Reducing Ability of Serum Assay

Nanoparticles ↙

Antioxidants in the serum sample

↓

Fe^{+++} ⟶ **Fe^{++}**

+

2,4,6-Tripyridyl-1,3,5-Triazine (TPTZ)

↓

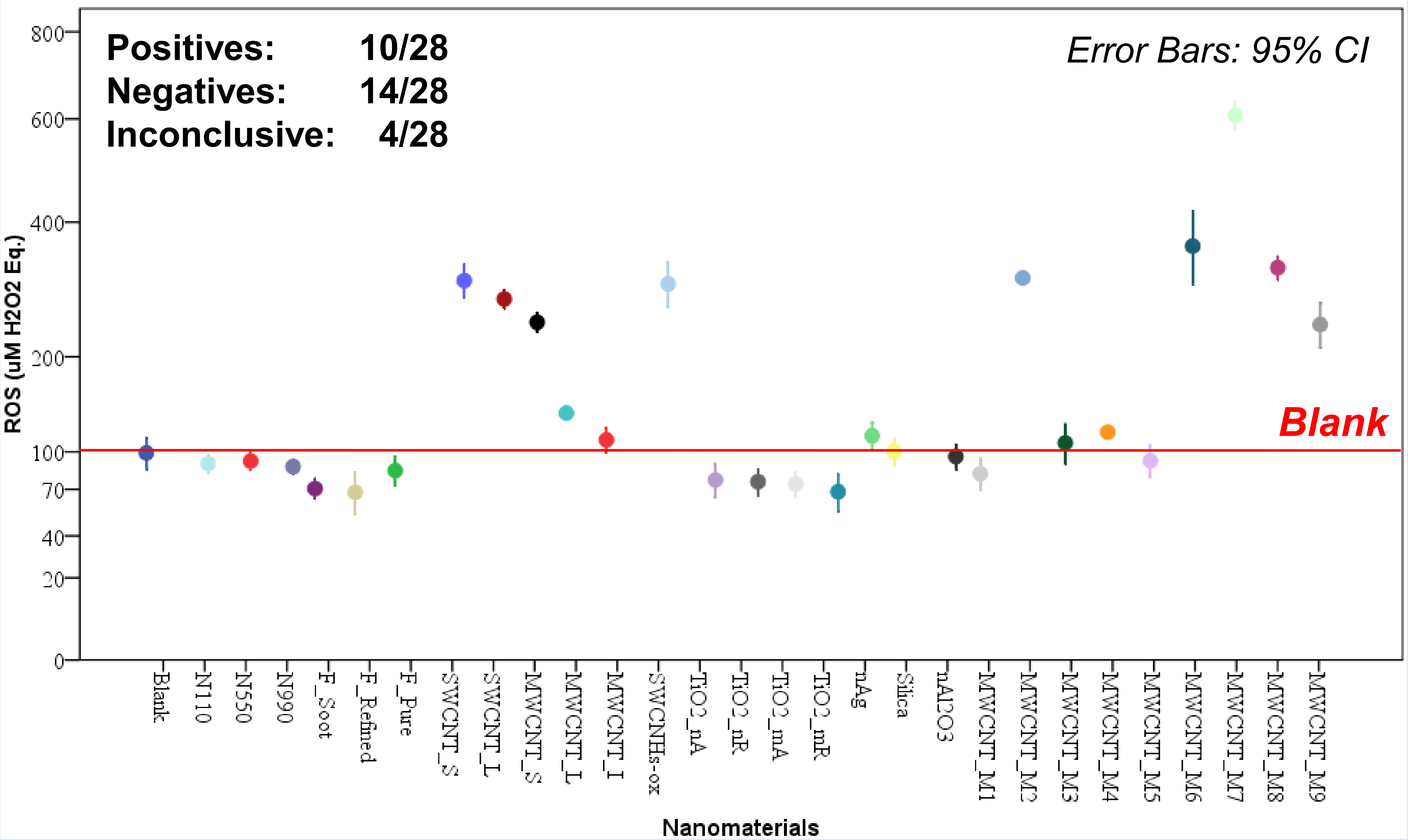
blue color

Decrease absorbance ⟶ Oxidant Damage

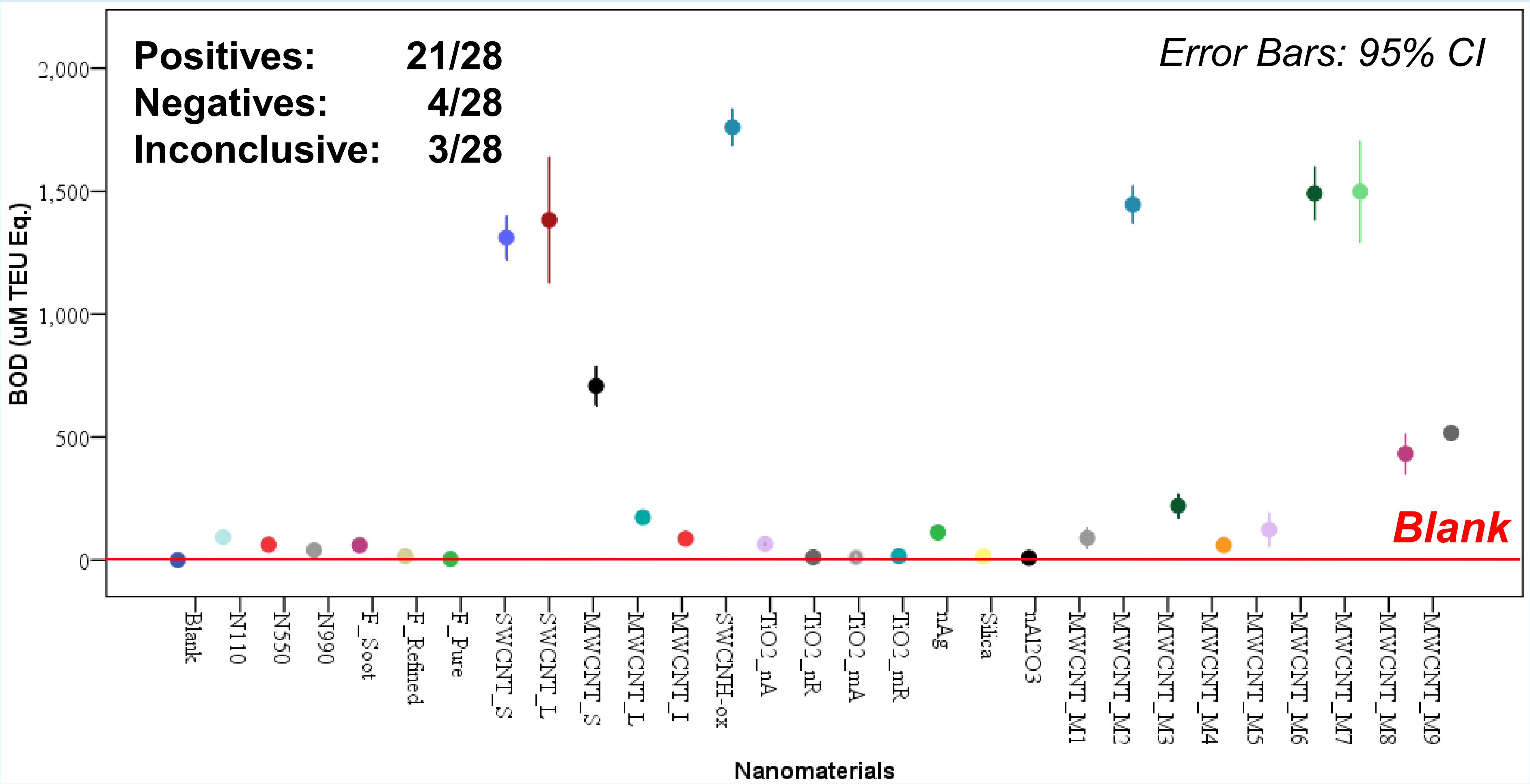
Standard Procedures of the FRAS Assay to Measure Oxidative Damage Induced by ENMs

1. Testing media – blood serum
2. Expose blood serum to selected ENMs (10mg mL^{-1}, 37°C, and 90 min)
3. Remove NPs by two step centrifugations (14,500 g for 15 min)
4. Measure antioxidant capacity of ENMs exposed serum by FRAS

DCFH Assay Results



FRAS Assay Results



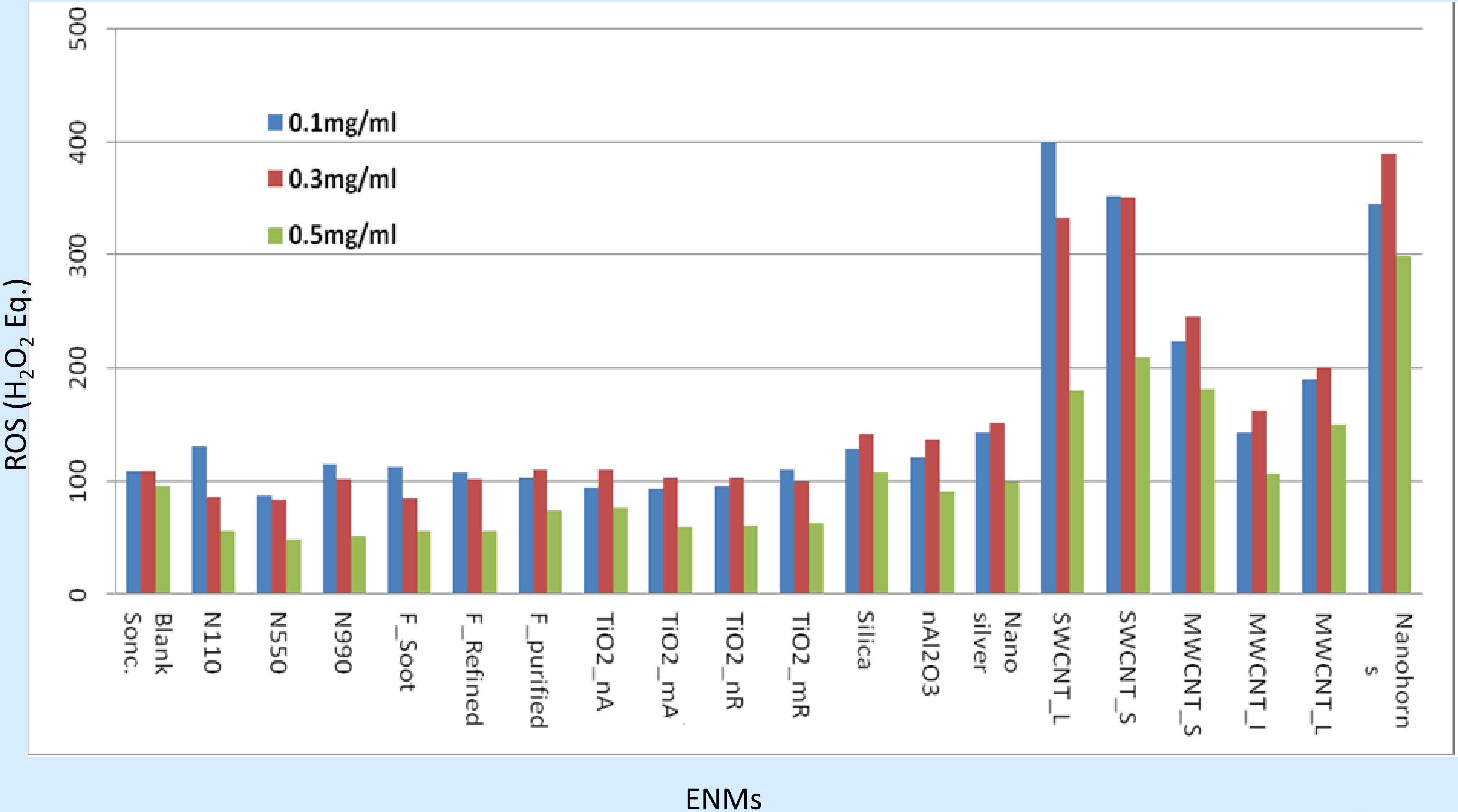
DCFH vs. FRAS: Comparison

		FRAS	
		Positives (21/28)	Negatives (7/28)
DCFH	Positives (10/28)	10	0
	Negatives (18/28)	11	7

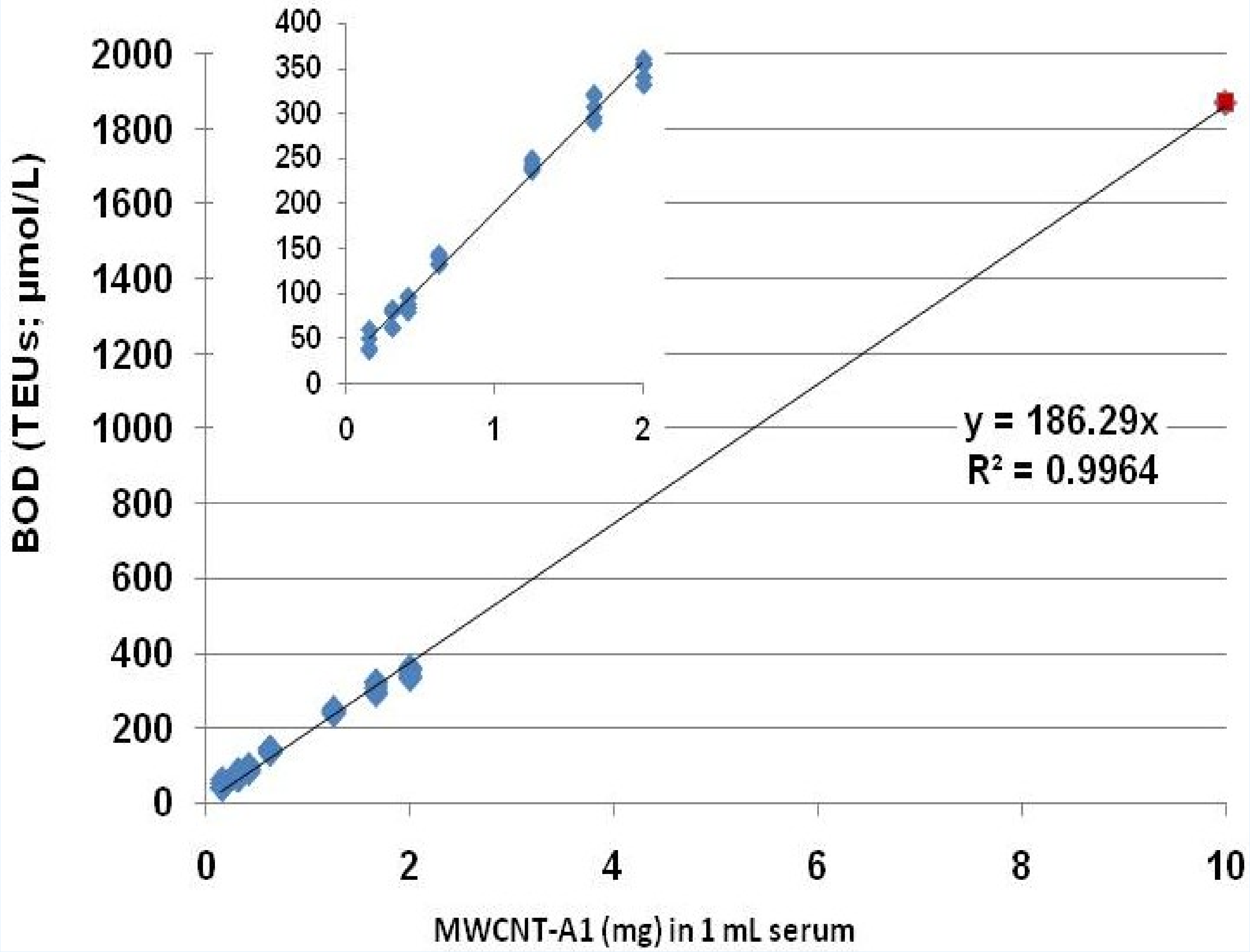
- FRAS gives positive result in every case DCFH does
- DCFH gives negative result in every case FRAS does
- FRAS never gives a negative result when DCFH gives a positive
- FRAS detects several positive results that DCFH fails to detect

→ ***FRAS has greater sensitivity across the board***

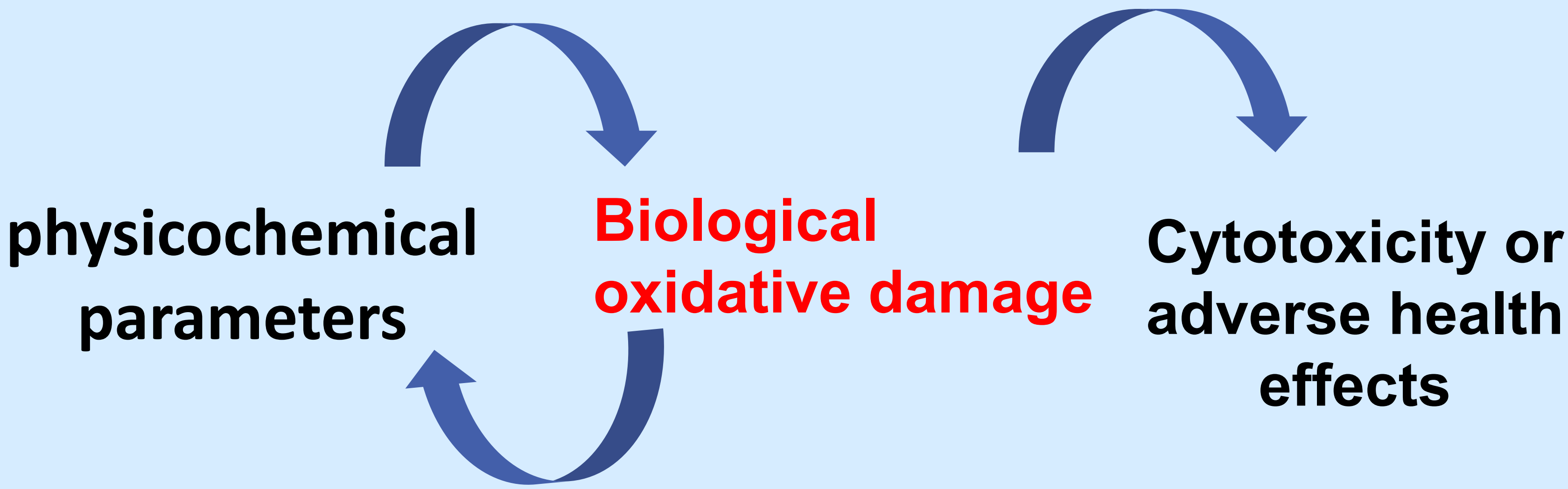
DCFH: Dose-Response



FRAS: Dose-Response



Linking Physicochemical Properties with Biology

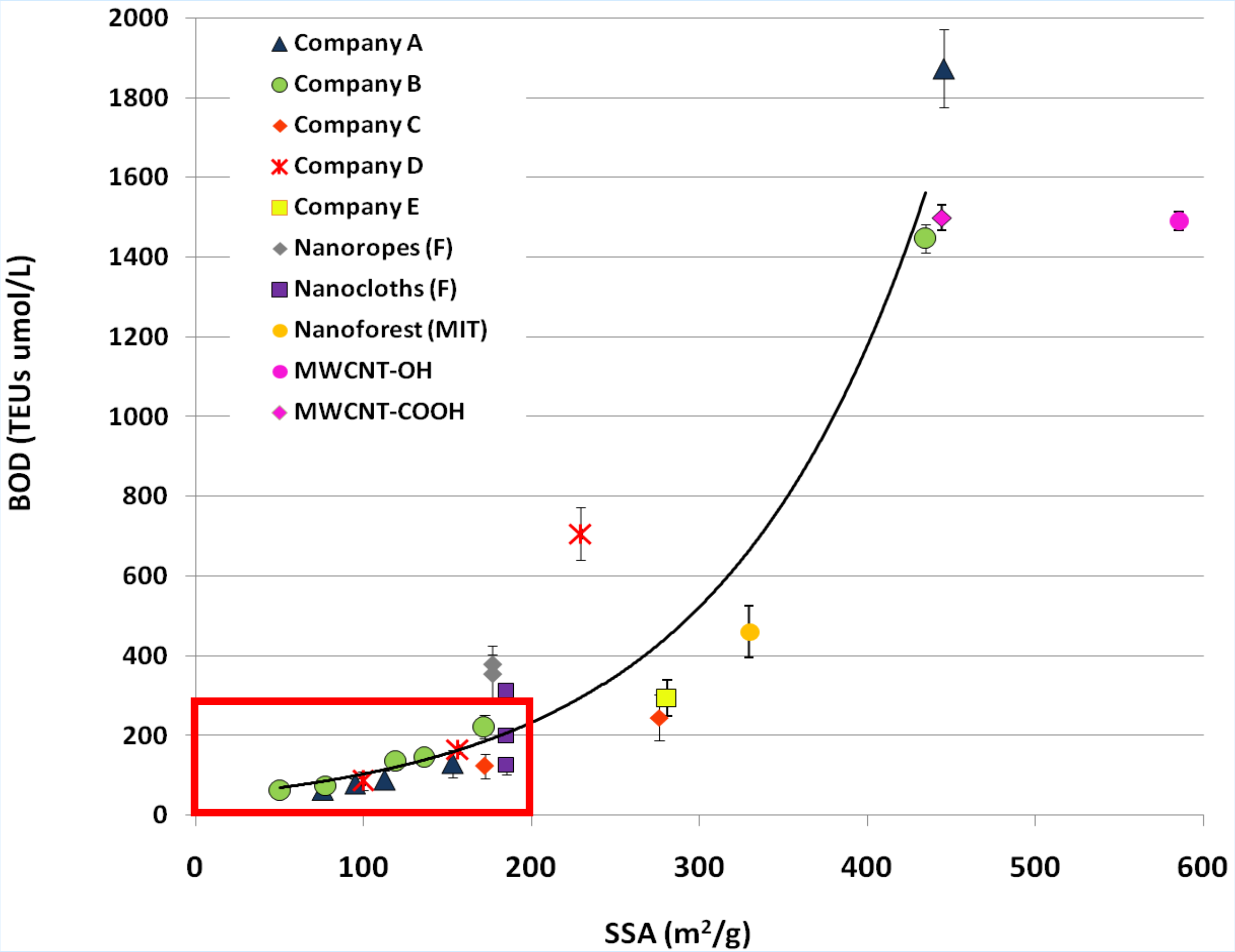


Standard Methods to Measure Physiochemical Properties of ENMs

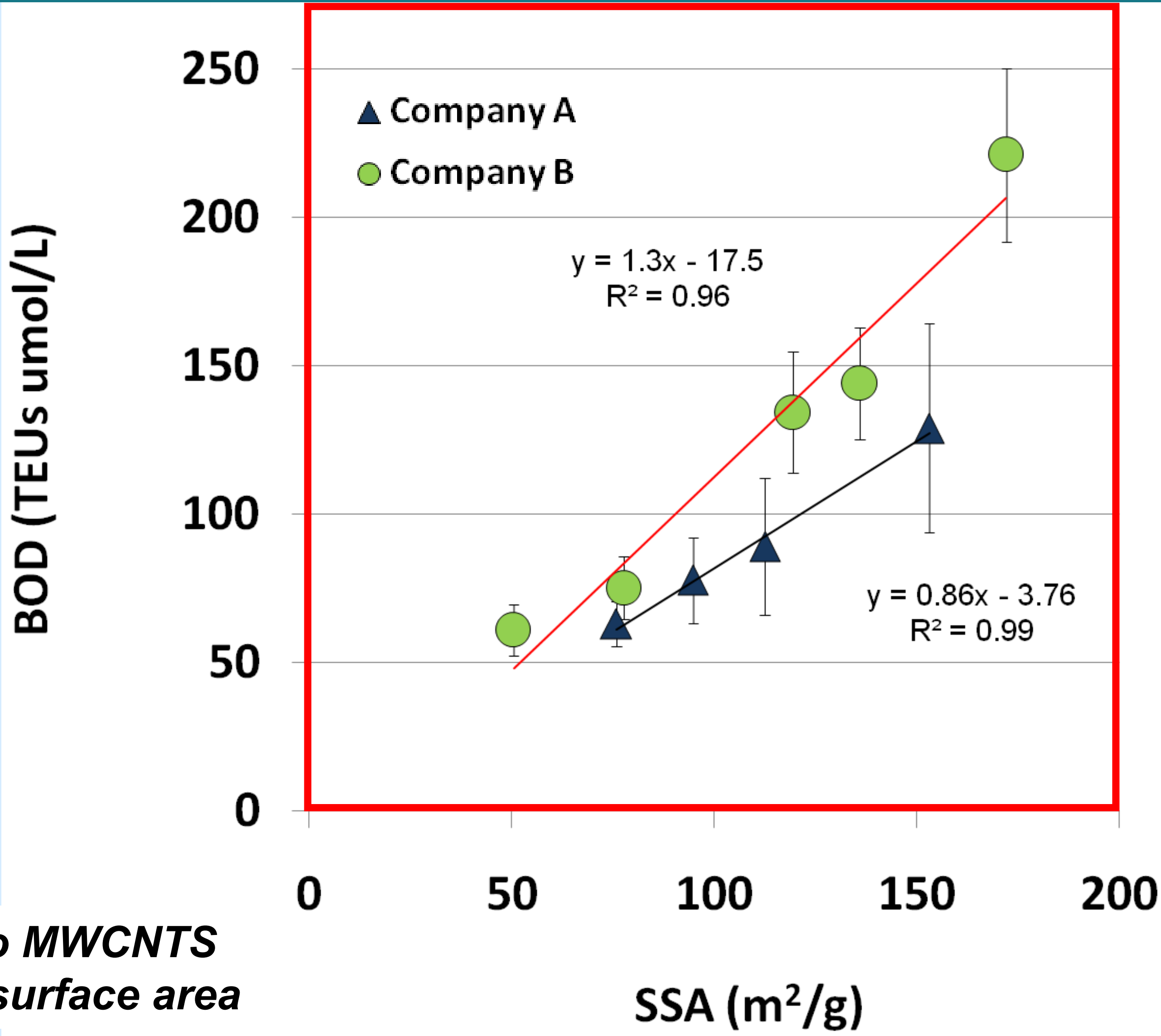
- **Surface area**
  - N2 sorption analysis (Quantachrome Autosorb-3B, 11-point BET)
- **Transition metals in bulk and water extract**
  - Instrumental Neutron Activation Analysis (INAA) and ICP-MS
- **Surface charge and mobility** - Zeta PALS
- **Crystallinity**- X-Ray diffraction
- **Morphology** - TEM & FE-SEM
- **Organic Carbon** – Modified NIOSH 5040
- **PAHs** - EPA method 3546 & GC-MS 8270

PAH-Polycyclic aromatic hydrocarbons

BOD Variations in MWCNTs



BOD Variations in MWCNTs



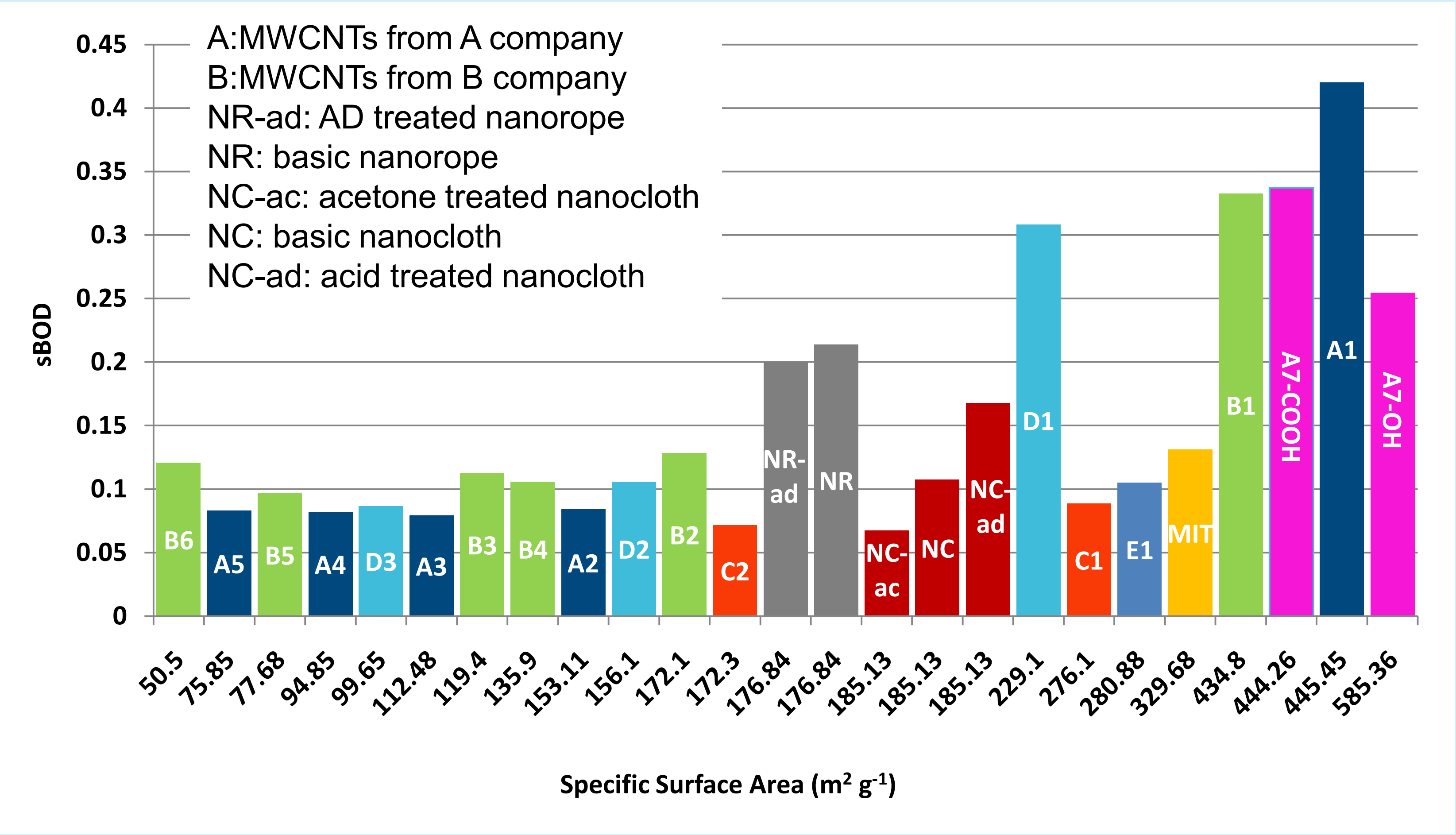
Excluded two MWCNTS having high surface area

Metal Distribution in MWCNTs

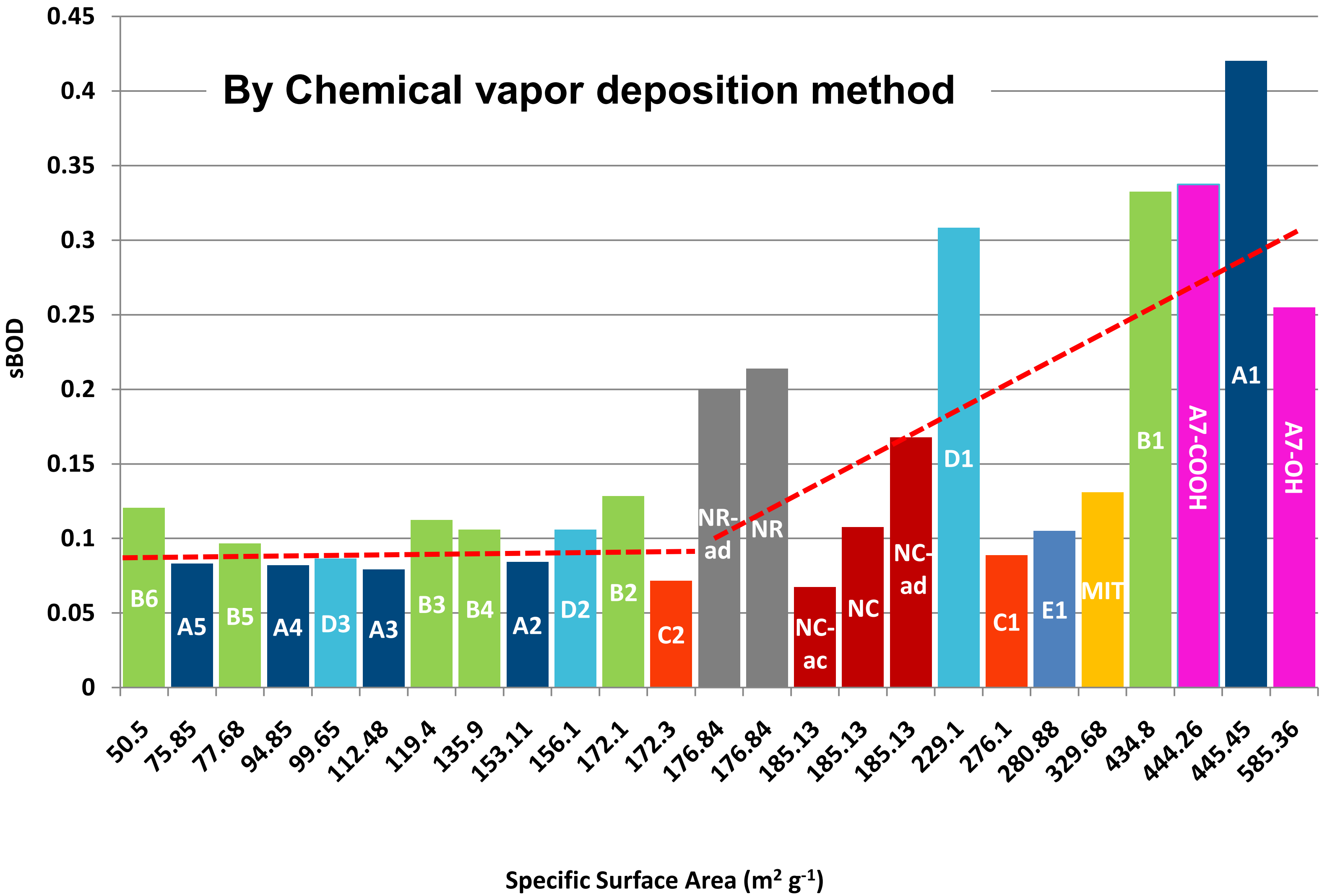
Material	BOD (TEUs, µmol/L)	SSA (m^2/g)	Fe	Ni	Co	Mo	Mn	La	Zn	Cr
MWCNT_A_1	1872	445.5	424	50	2075.1	1182	12.4	5.3	109	67.4
MWCNT_B_1	1445	434.8	164	25.9	1150	714	3.2	2.4	15.9	40.2
MWCNT_A_2	129	153.1	2003	2946	28.5	37.1	20.5	1022	98.9	23.9
MWCNT_B_2	221	172.1	227	3172	12.7	20.5	4.7	270	24.3	20.4
MWCNT_A_3	89.1	112.5	1724	6258	3.6	6.5	6.4	109	54.8	22.2
MWCNT_B_3	134	119.4	3905	8863	115	46.7	29.8	427	18	98
MWCNT_A_4	77.7	94.9	1931	2766	19	18.6	26.4	84.1	65.8	21
MWCNT_B_4	144	135.9	269	1191	0.8	25.3	1.5	408	16.9	4.5
MWCNT_A_5	62.9	75.9	269	5564	11.9	19.5	63.4	31.9	37.8	51.8
MWCNT_B_5	75.0	77.7	496	7888	7.5	18.6	4.7	167	15.1	11.3
MWCNT_B_6	60.9	50.5	144	5057	56.4	341	1.5	423	28.3	4.3
MWCNT_C_1	244	276.1	9759	4.3	0.5	5945	0.6	<0.4	23.3	1.4
MWCNT_C_2	123	172.3	14780	3.2	0.6	5589	2.6	<0.3	1383	1
MWCNT_D_1	706	229.1	172	4737	68.9	112	5.32	103	11.8	9.99
MWCNT_D_2	165	156.1	0.00	5890	0.00	155	0.00	64.1	2.89	0.00
MWCNT_D_3	86.3	99.7	496	51866	120	191	25.8	20.5	8.55	33.0
MWCNT-OH	1491	585.4	195	19.5	1214	4887	1547	16.5	13.4	53.8
MWCNT-COOH	1498	444.3	124	10.4	644	349	1.3	13.4	6.3	28.9
Nanoforest I (assay 1)	432	329.7	712	<9.6	<9.6	173	<9.6	<9.6	294	<9.6
Nanoforest I (assay 2)	432	329.7	277	<8.5	<8.5	61.8	<8.5	<8.5	278	<8.5
Nanoforest III	517	329.7	396	29.7	<8.8	26.1	<8.8	<8.8	644	<8.8
Nano-rope (F_1)	353	176.8	46961	27.8	1.4	18.3	1	<1.0	350	5
Nano-cloth (F_2)	294	280.9	27	0.7	60.5	2455	<0.3	<0.3	8.2	<0.3
Correlation to BOD			-0.1	-0.3	0.9**	0.2	0.4	-0.3	-0.1	0.4
Correlation to sBOD			-0.02	-0.2	0.8**	-0.02	0.2	-0.3	-0.2	0.3

Fe-iron
Cr-Chromium
Co-Cobalt
Mo-Molybdenum
Mn-Maganese

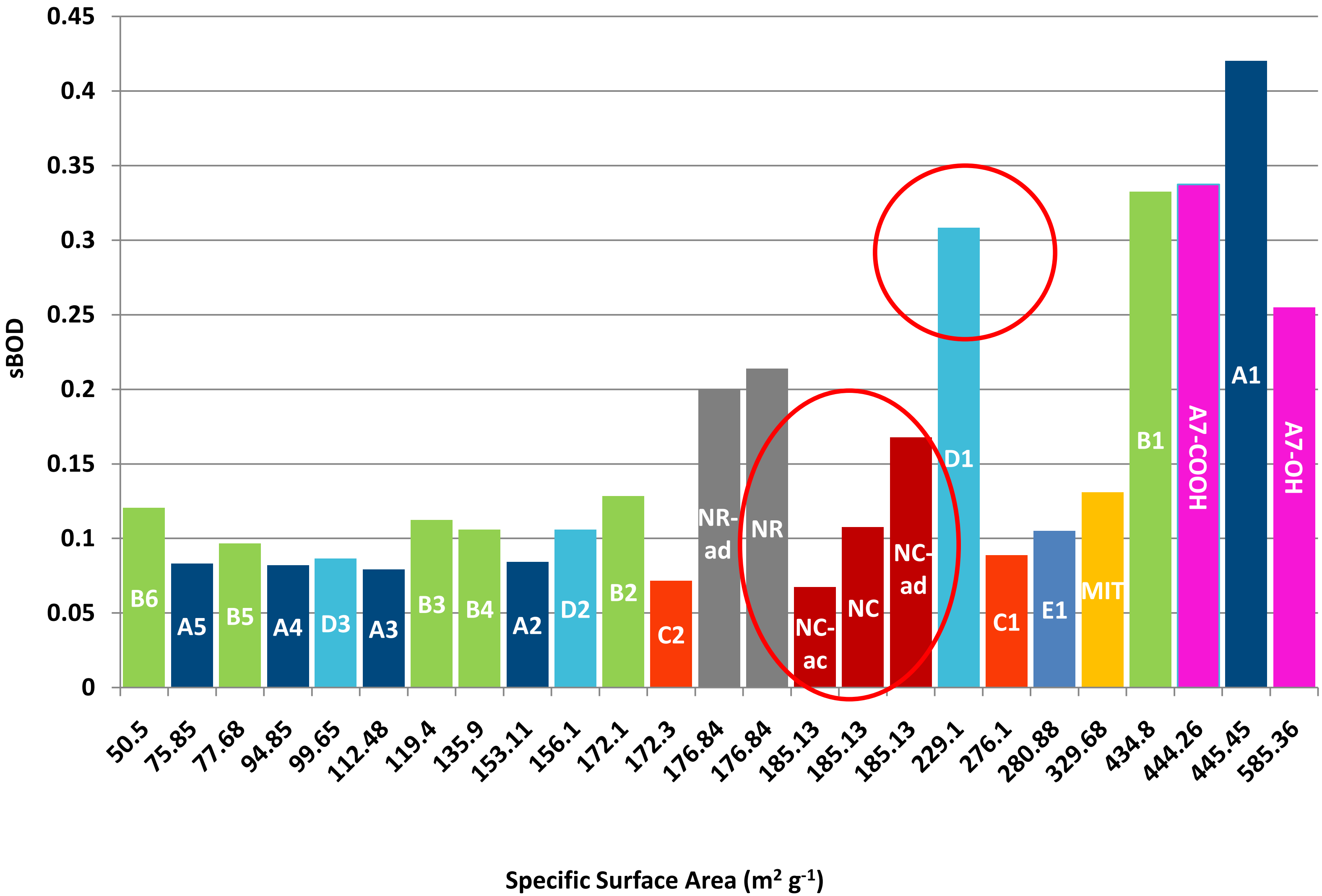
List of Standardized Biological Oxidant Damage (sBOD) by Specific Surface Area



sBOD represent BOD induced by one unit surface was calculated as degree of BOD (µmol of trolox equivalent units) generated by one unit surface area (m^2) of MWCNT in 1 ml exposed serum.



By Chemical vapor deposition method
sBOD
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
B6
A5
B5
A4
D3
A3
B3
B4
A2
D2
B2
C2
NR-ad
NR
NC-ac
NC
NC-ad
D1
C1
E1
MIT
B1
A7-COOH
A1
A7-OH
50.5
75.85
77.68
94.85
99.65
112.48
119.4
135.9
153.11
156.1
172.1
172.3
176.84
176.84
185.13
185.13
185.13
229.1
276.1
280.88
329.68
434.8
444.26
445.45
585.36
Specific Surface Area (m2 g-1)


0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
sBOD
B6
A5
B5
A4
D3
A3
B3
B4
A2
D2
B2
C2
NR-ad
NR
NC-ac
NC
NC-ad
D1
C1
E1
MIT
B1
A7-COOH
A1
A7-OH
50.5
75.85
77.68
94.85
99.65
112.48
119.4
135.9
153.11
156.1
172.1
172.3
176.84
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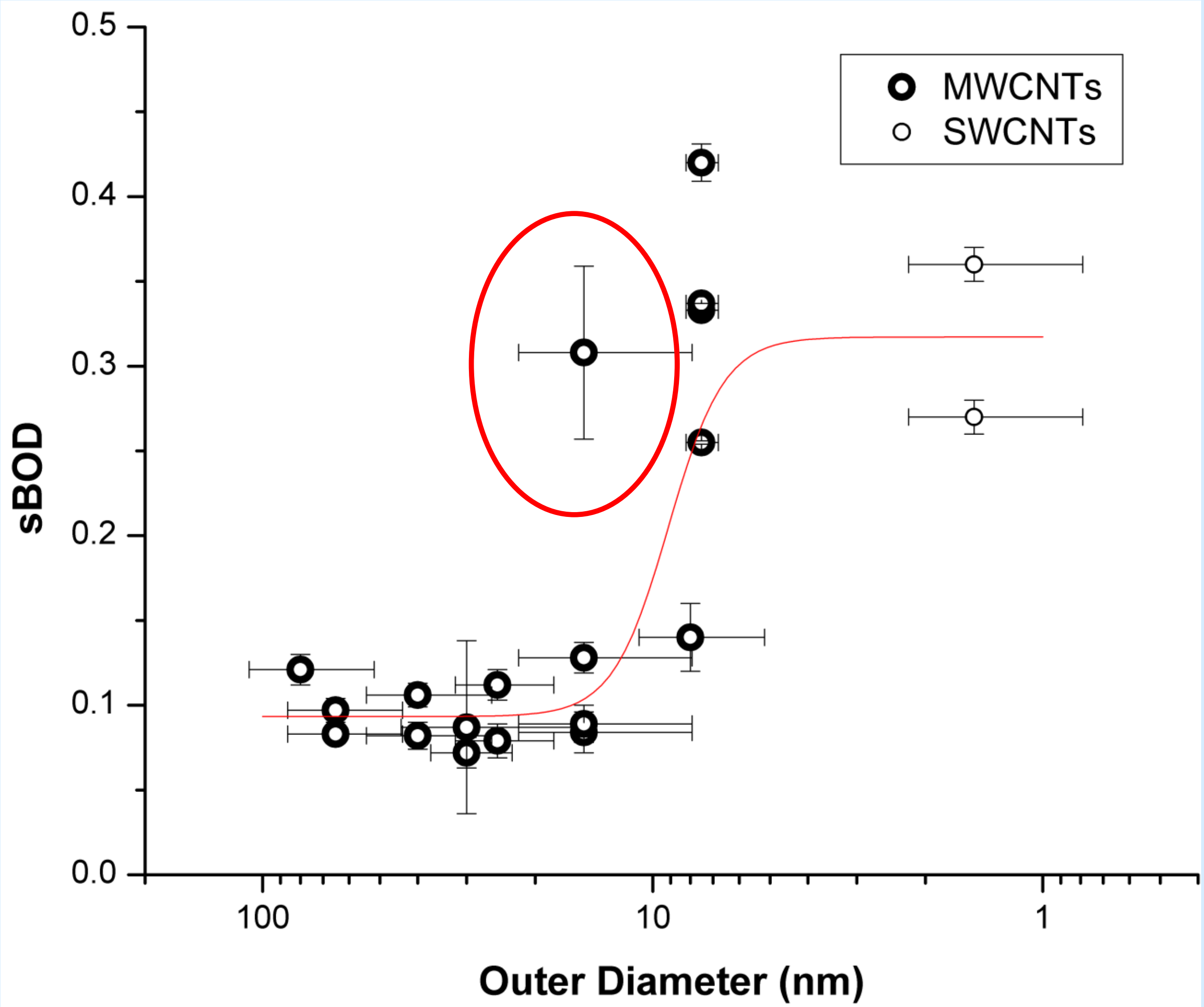
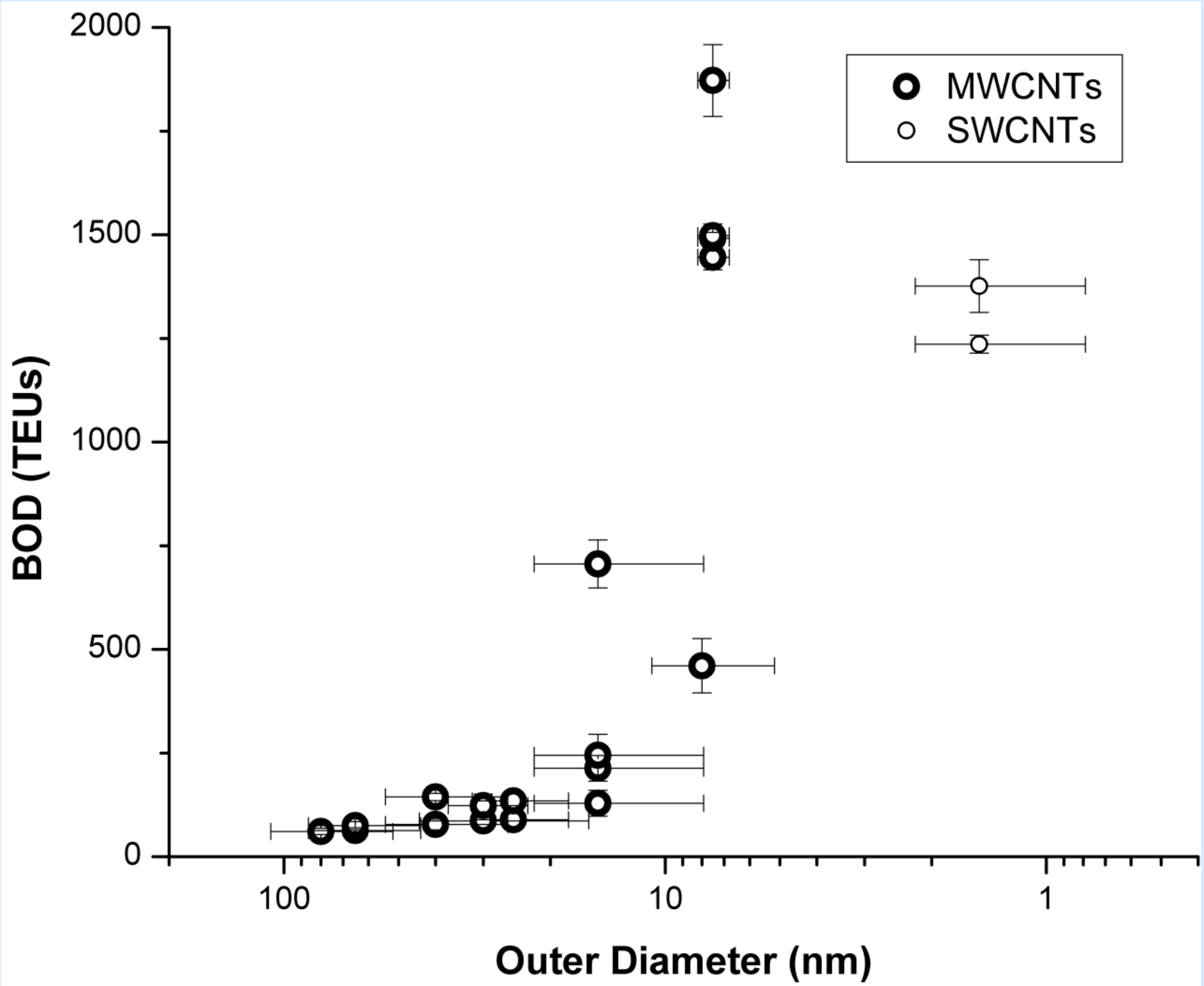
Specific Surface Area (m^2 g^{-1})

Surface reactivity of CNTs

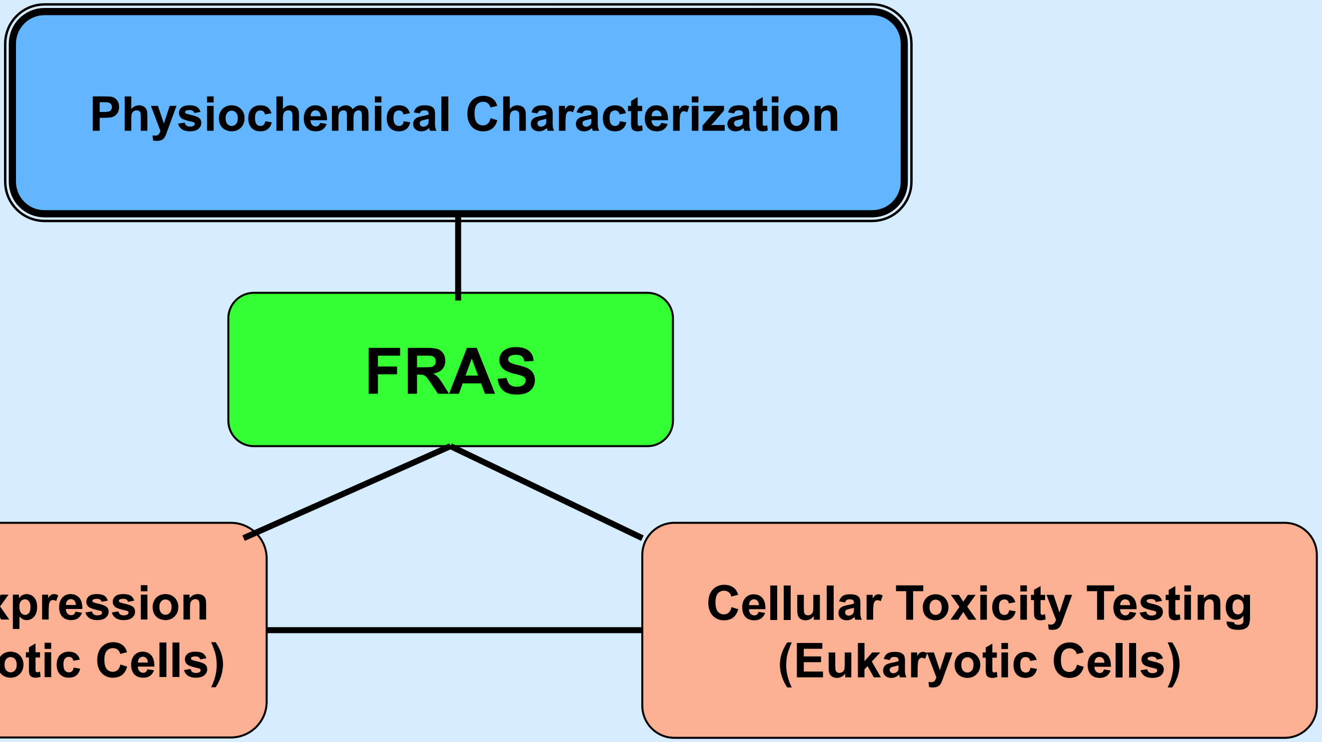


Surface reactivity of CNTs



Path Forward

Linking Physicochemical Properties with Biology



Acknowledgments

- **This work was supported by the National Science Foundation as a Nanoscale Science and Engineering Centers Program (Award # NSF-0425826)**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