Cytotoxic effects of engineered nanoparticles at the bio-nano interface

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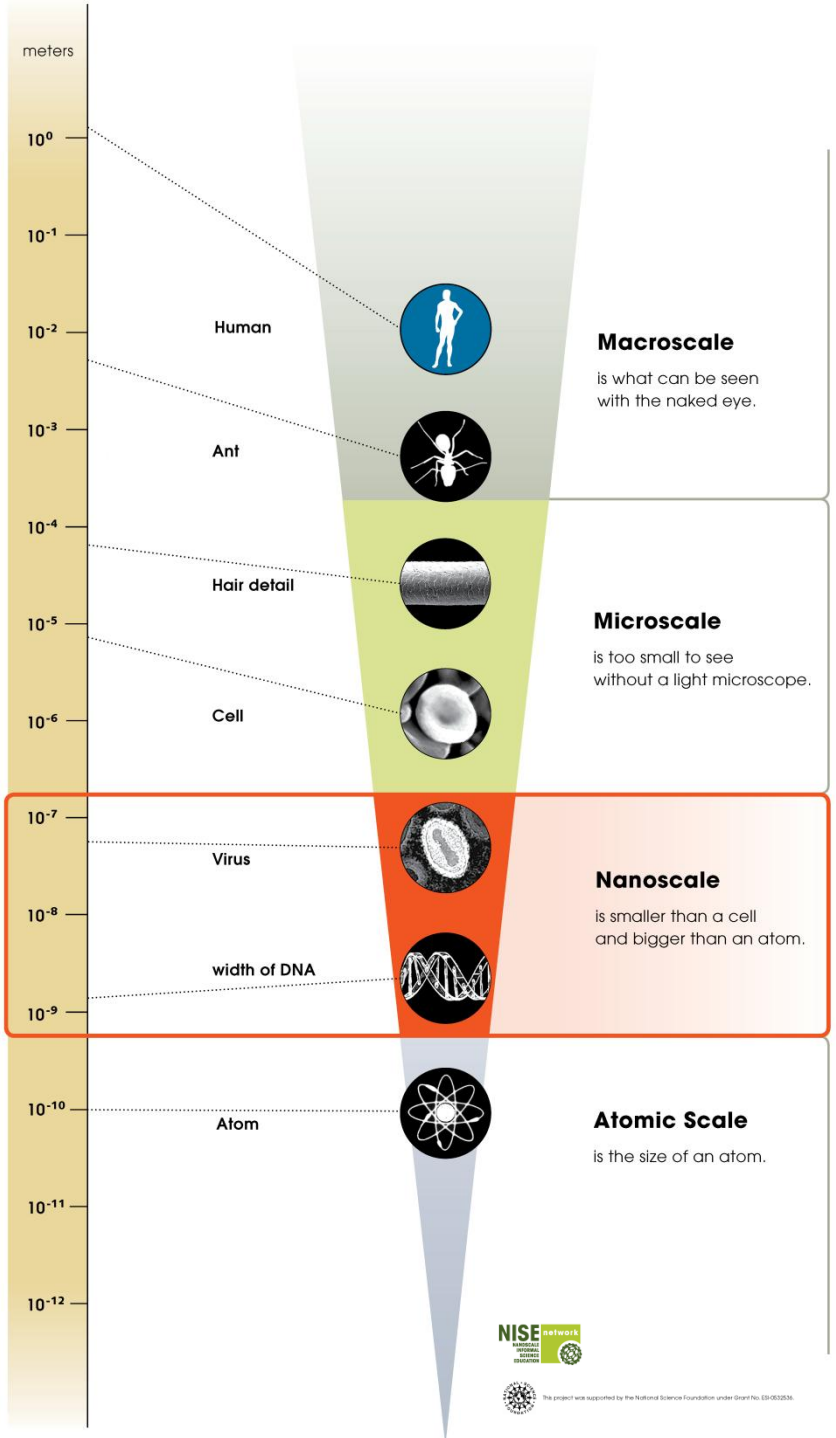
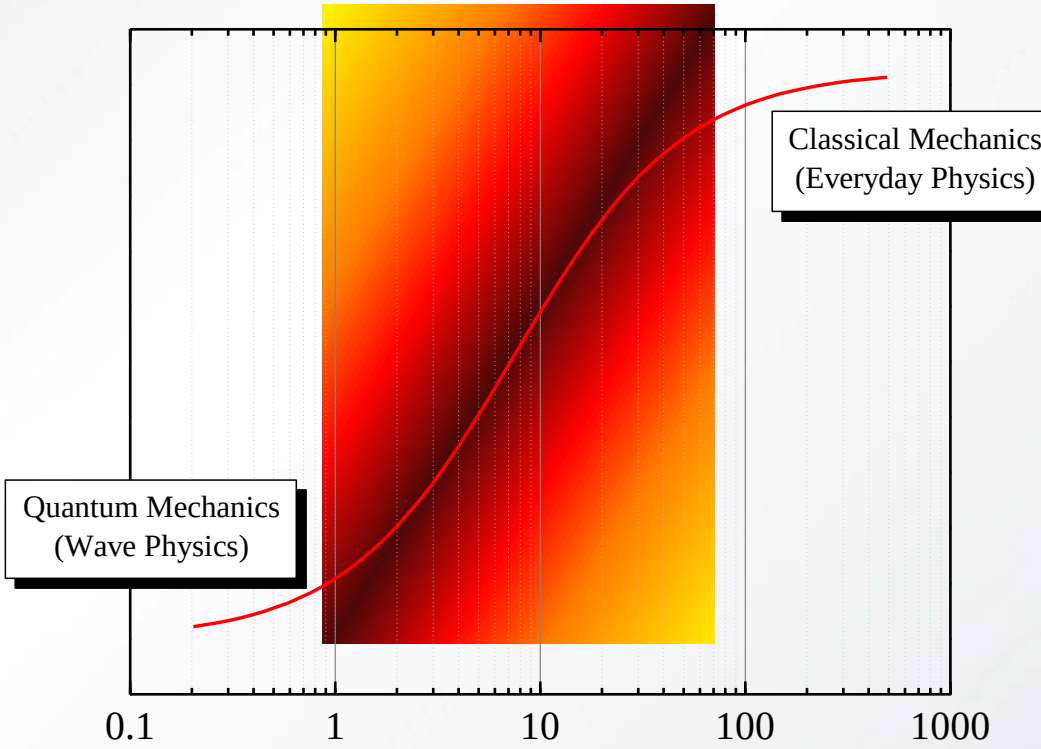
Georgia Institute of Technology

School of Civil and Environmental Engineering

What happens at nanoscale?





- High specific surface area
- High reactivity
- High transportability
- More……

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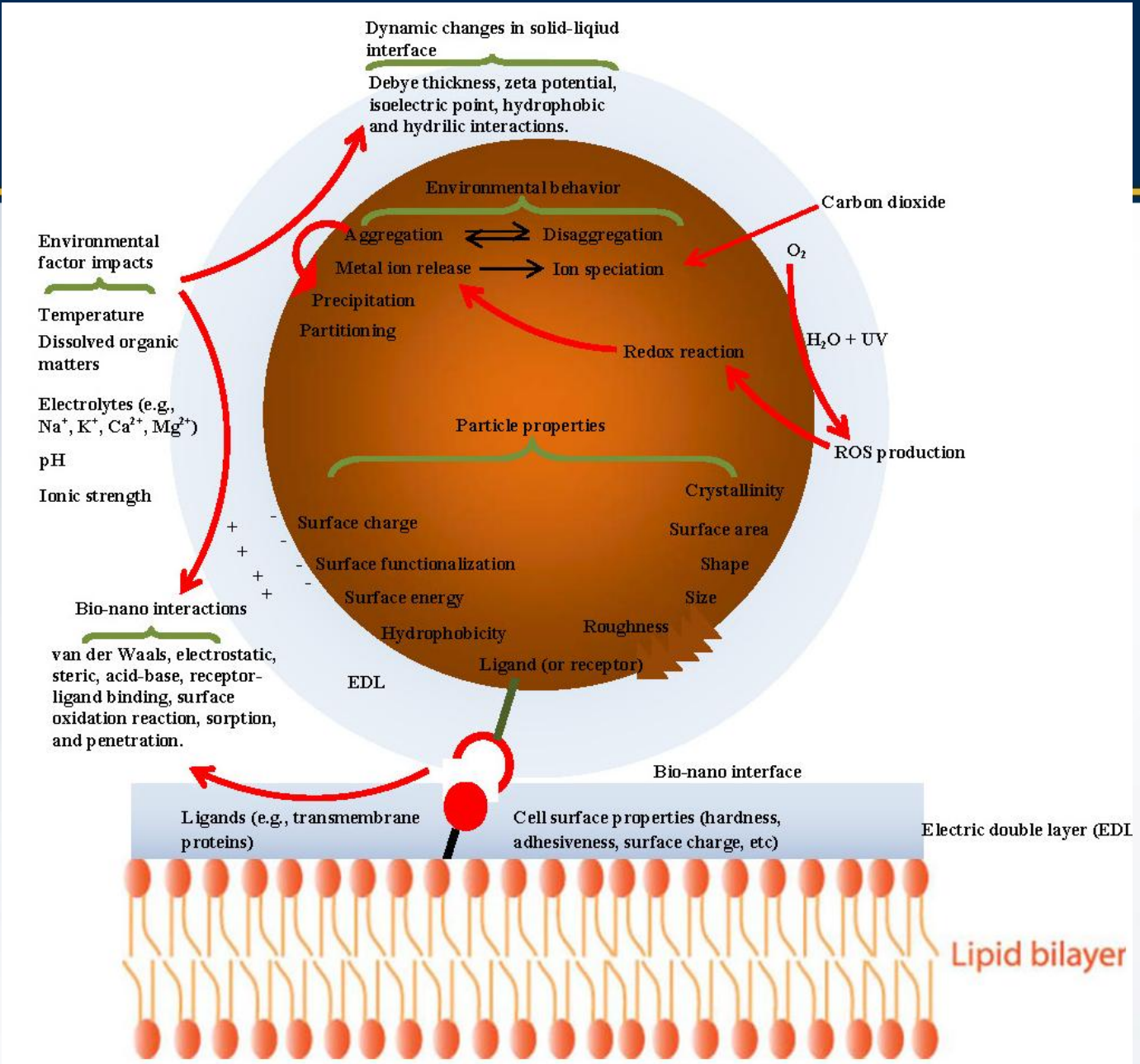

Figure 1.1. Representation of the interface between a nanoparticle and an intact lipid bilayer representative of a cell surface. Various environmental factors, particle properties, and their interrelationships are depicted. Credit: Wen Zhang. PhD thesis, Georgia Tech. 2011

What could happen to NPs at biological interface?

- Adsorption
- Specific (ligand/receptor) and non-specific interactions
- Disruption (e.g., physical and chemical damage)
- Permeation/penetration

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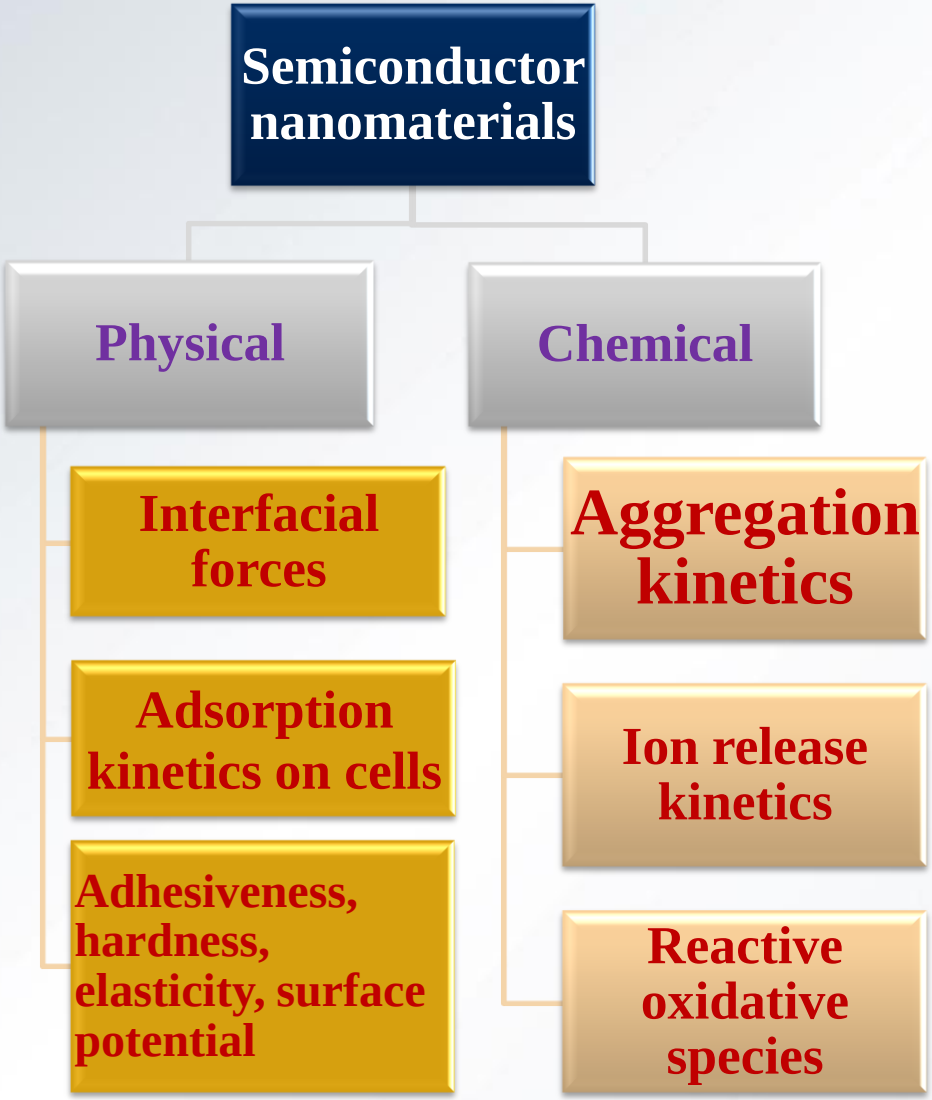
My research outline



Fe_2O_3
CeO_2
TiO_2
ZnO
Al_2O_3
CuO
SiO_2
QDs
Au
Ag

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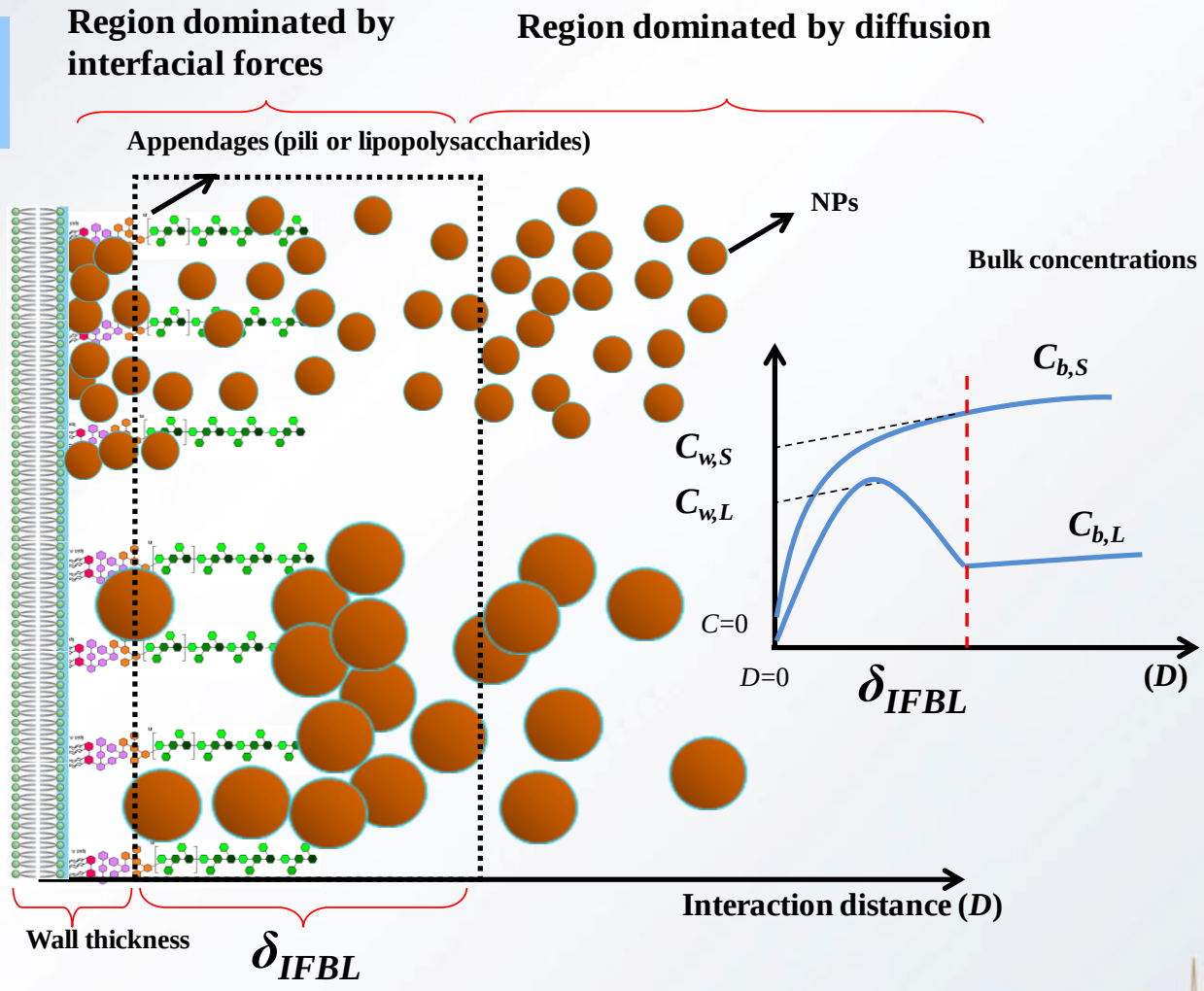
Some of the key findings on adsorption kinetics and the associated cellular impairments

Interaction force boundary layer (IFBL) model

- **Role of random kinetic energy (diffusivity)**
- **Role of interfacial energy**
- **Adsorption kinetics (i.e., rate constant)**



Wen Zhang, Bruce Rittmann, and Yongsheng Chen. **Size effects on adsorption kinetics of hematite NPs on *E. coli* cells**. ***Environmental Science and Technology***, 2011, 45 (6), 2172-2178.

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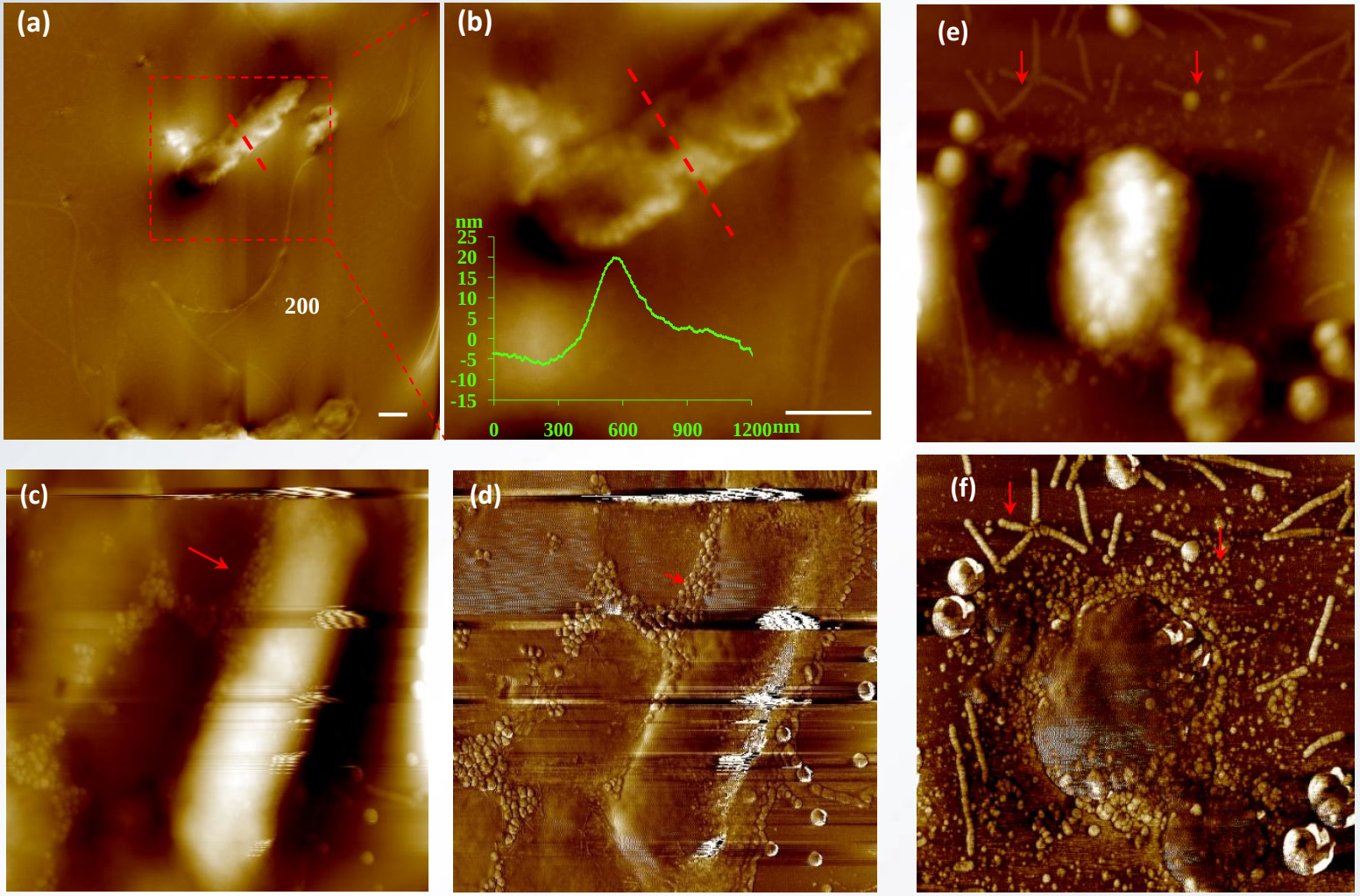
Surface disruption to bacteria (*E. coli*) after exposure to hematite NPs



Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of Hematite Nanoparticle Exposure on Biomechanical and Surface Electrical Properties of E. coli Cells. ***Applied environmental microbiology (AEM)***, submitted.

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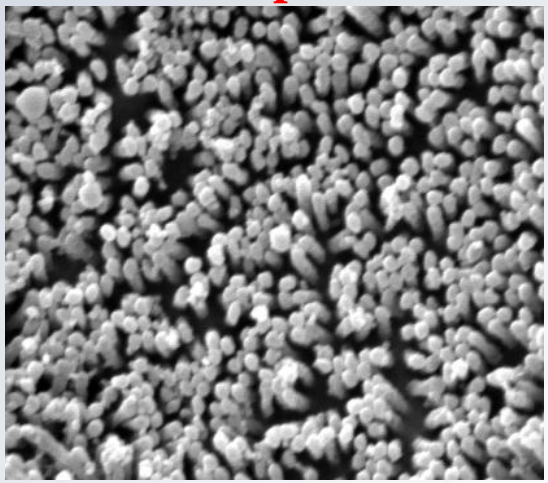
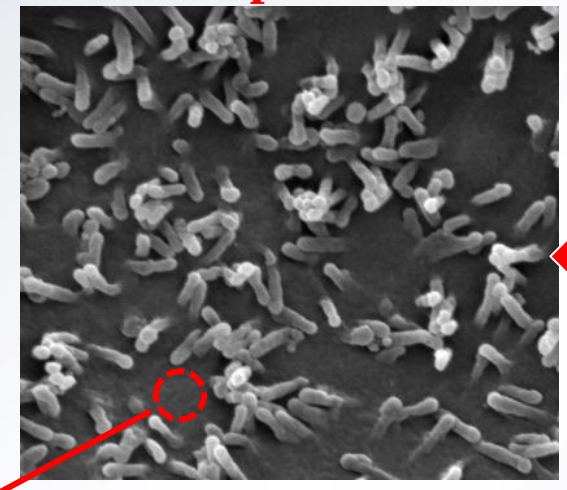
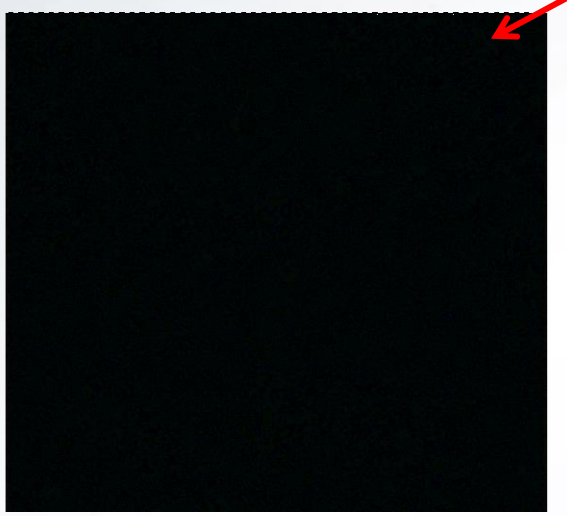
Surface disruption to human intestinal cells (Caco-2) after exposure to hematite NPs

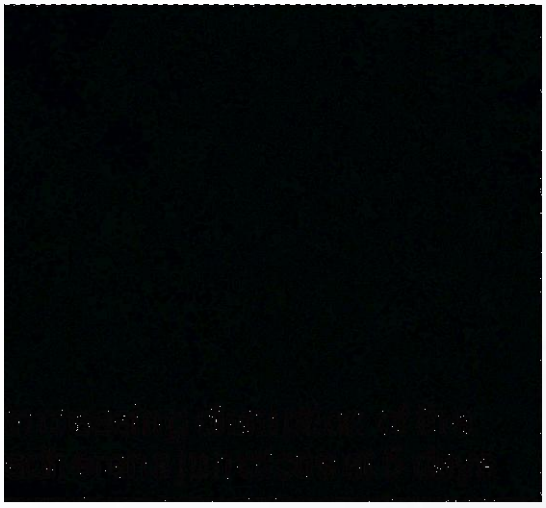
Before exposure

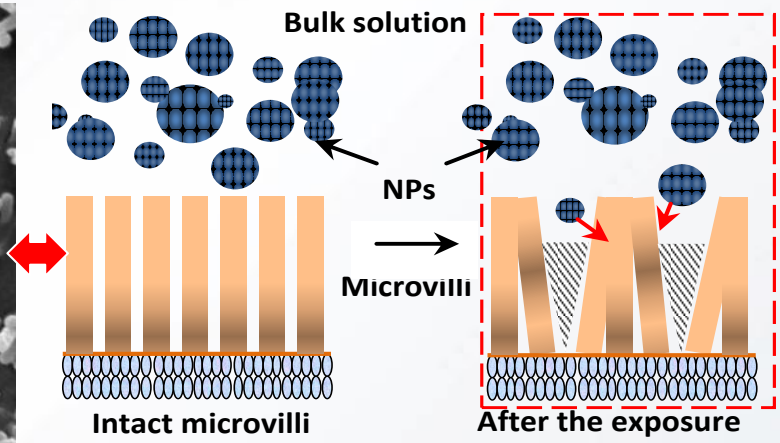


After exposure









- **Microville disruption, membrane penetration, and adheren junction disruption.**
- **Possibly interpreted by depletion attraction.**

Wen Zhang, Madhavi Kalive, David G Capco, and Yongsheng Chen, Adsorption of hematite nanoparticles onto Caco-2 cells and the cellular impairments: effect of particle size. ***Nanotechnology*** 2010, 21, 355103.

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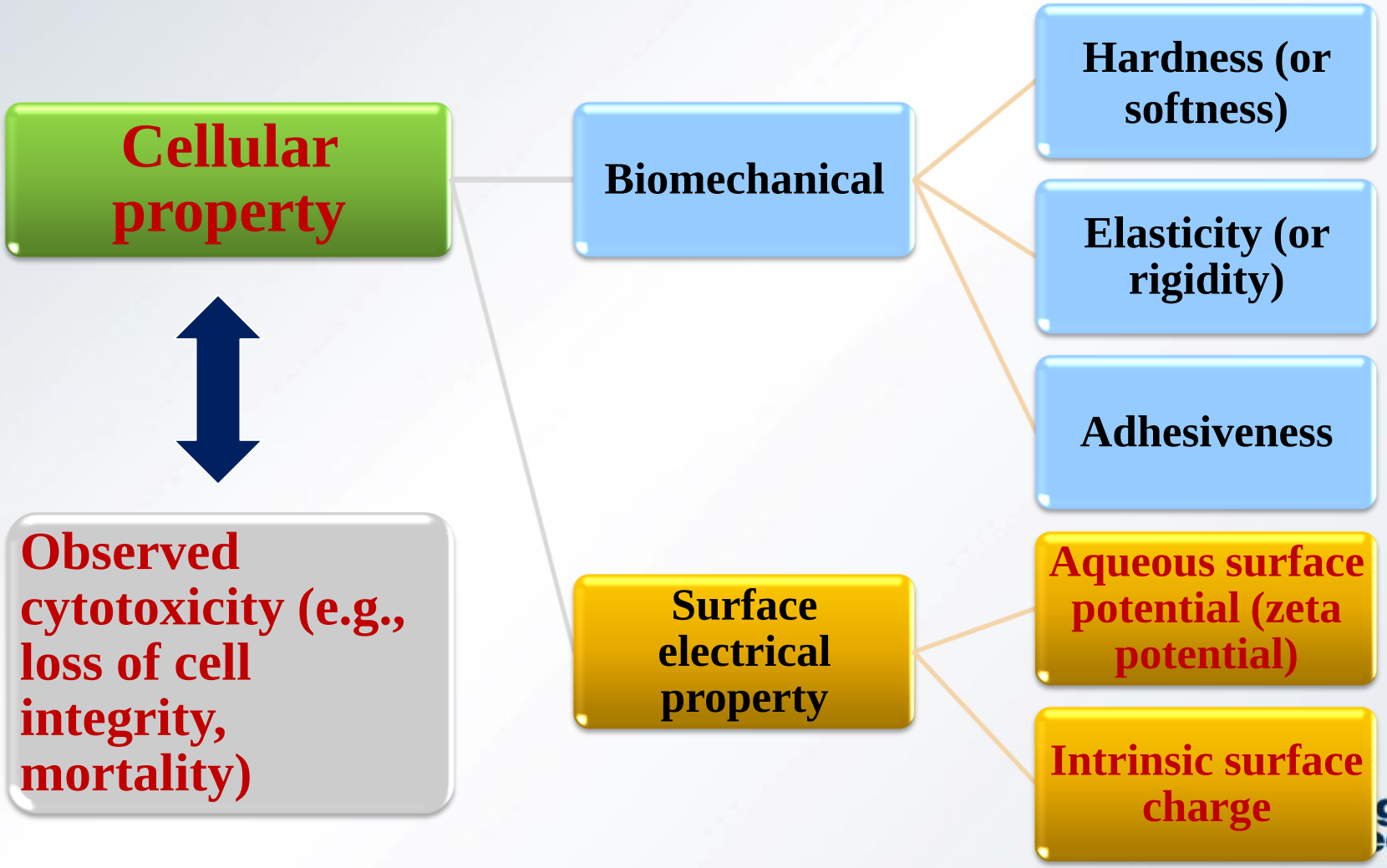
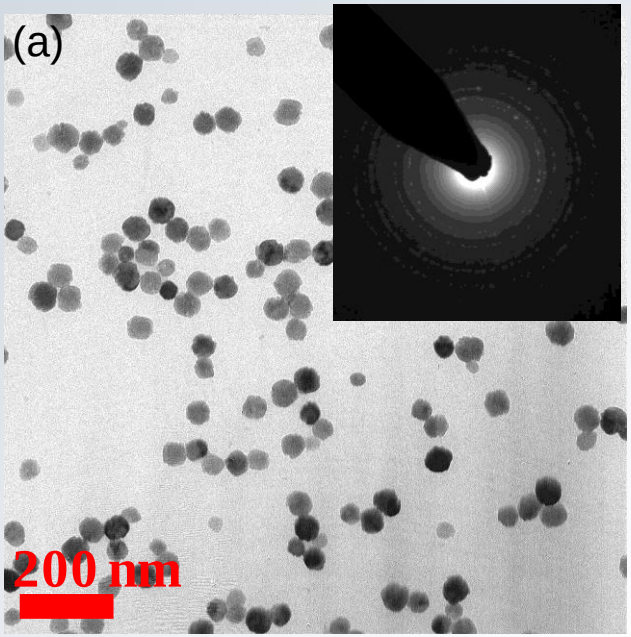
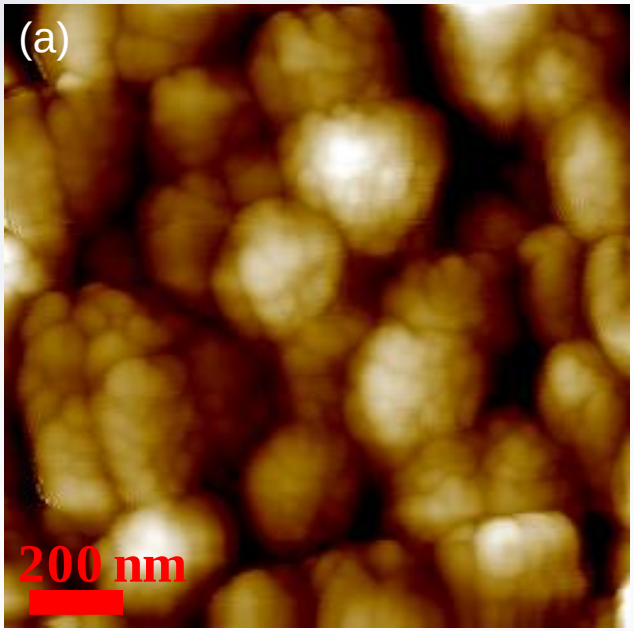
Mechanisms of surface disruption from a physical perspective: Fe_2O_3 vs *E. coli*

To better understand and interpret nanotoxicity test results:

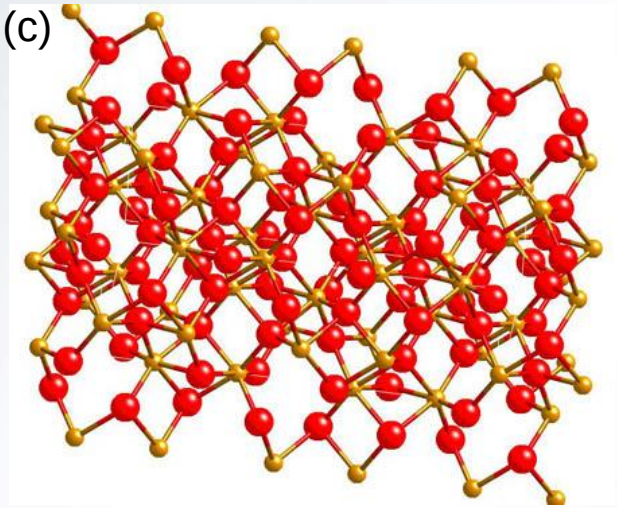


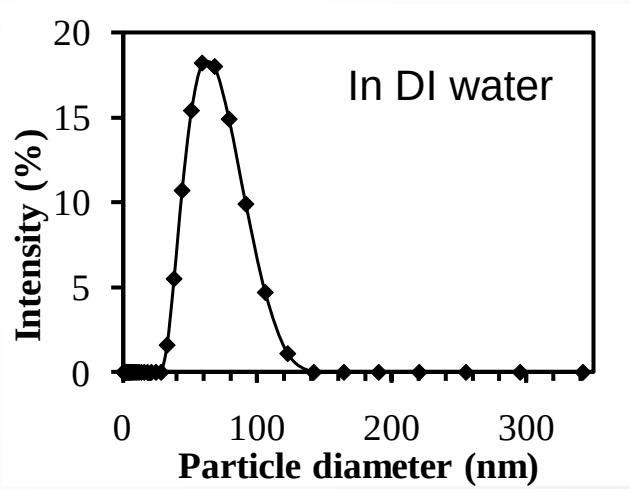
Mechanisms of surface disruption from a physical perspective: Fe_2O_3 vs *E. coli*





(a) TEM image of hematite (α-Fe_2O_3) NPs with inset of SAED pattern; (b) AFM image of aggregated clusters of hematite NPs; (c) Crystal structure of hematite NPs; and (d) The size distribution





Several important features of hematite (reasons of choosing it in the test)

- ✓**Good reference nanomaterial**
- ✓**Good aqueous stability**
- ✓**Relatively uniform size distribution**
- ✓**No toxic metal release (good chemical stability or chemically inert)**

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Mechanisms of surface disruption from a physical perspective: Fe_2O_3 vs *E. coli*

Reprehensive morphology of *E. coli* cells

Several important advantages by using *E. coli* as a cell target

- ✓One of the basic and representative Gram-negative bacterial form that mirrors most of the bacterial properties
- ✓Rapid toxicity screening relative to human cell lines
- ✓A representative microorganism widely used in nanotoxicity tests
- ✓Commonly used experimentally in molecular biology
- ✓Comprehensive knowledge of gene library that allows us to conduct future genetic level studies

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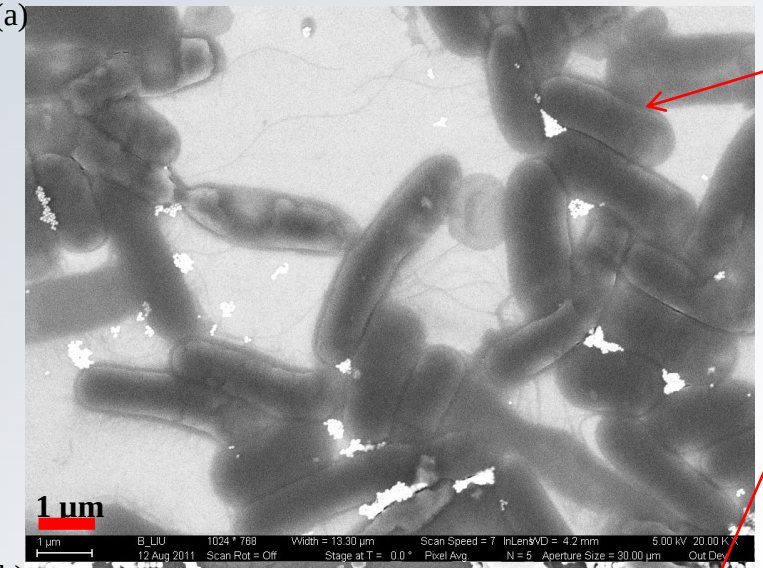
Results

1. Morphology changes of *E. coli* after exposure to hematite NPs

(a)

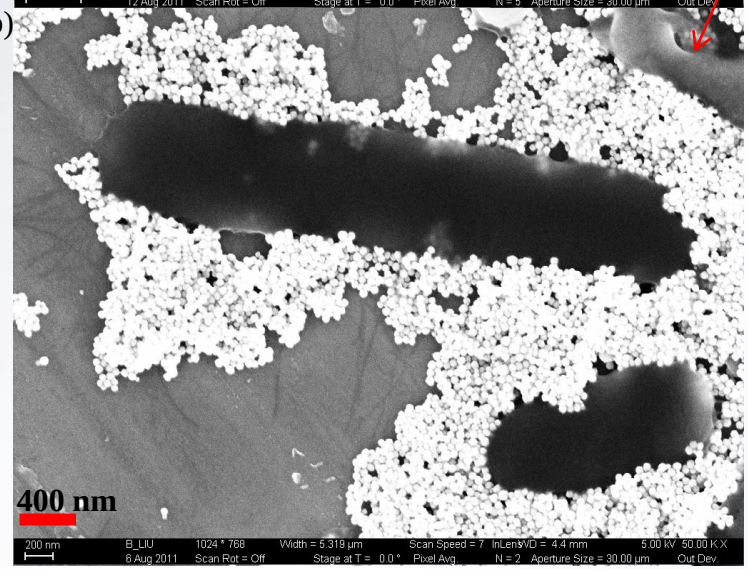


E. coli **cells with a few hematite NPs (white dots) attached**

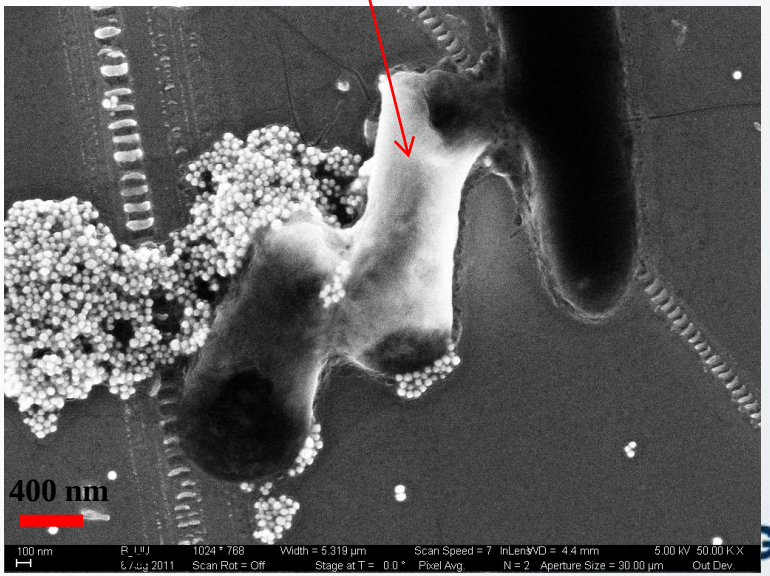
E. coli **cells with heavy adsorption of hematite NPs and aggregated clusters**

Deformed *E. coli* cells after long time exposure to hematite NPs

(b)



(c)



Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.

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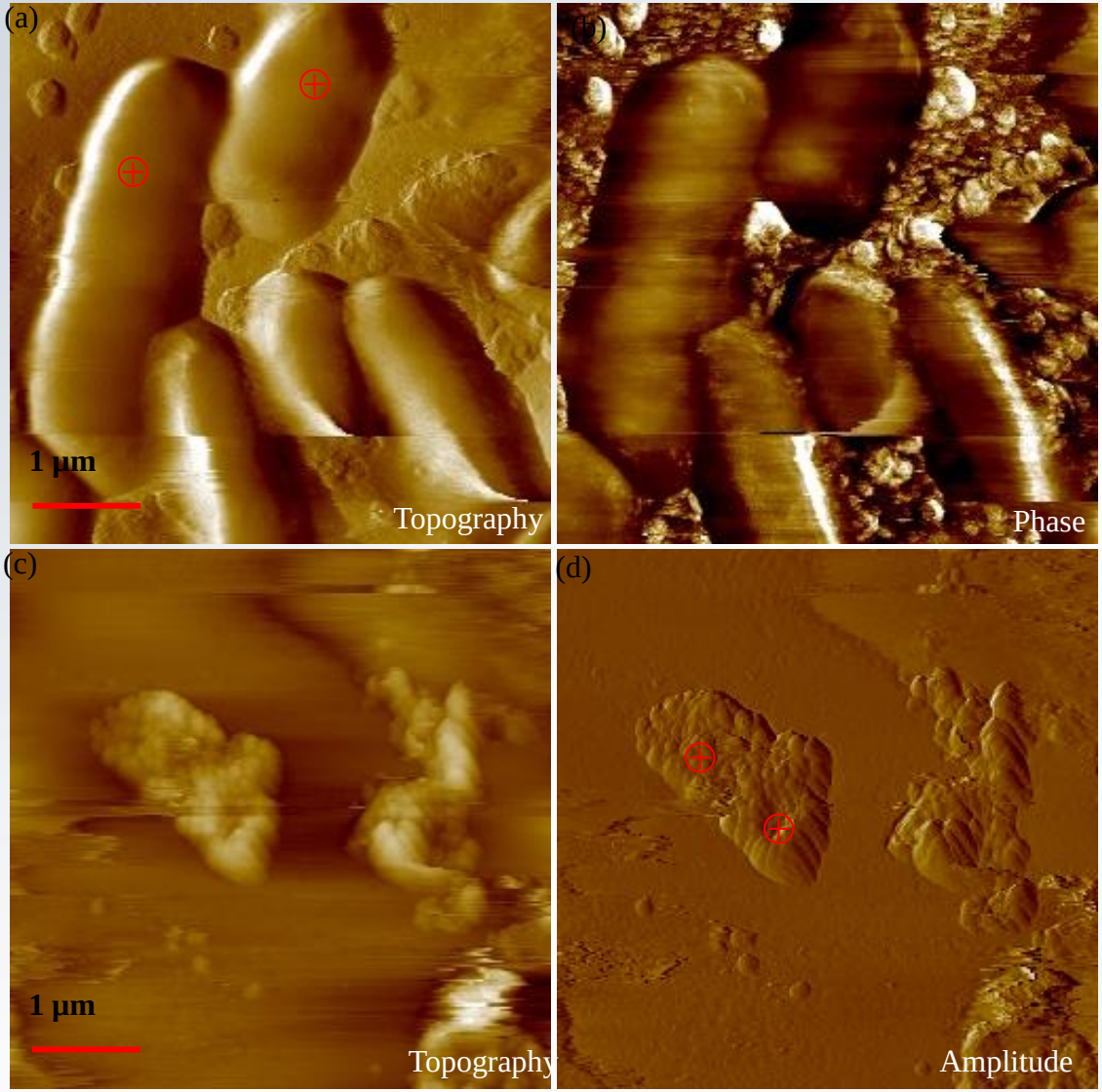
Results
1. Morphology changes of *E. coli* after exposure to hematite NPs



Quasi-in situ imaging of *E. coli* cells in liquid by AFM.

- *E. coli* cells immobilized on a freshly cleaned silicon wafer surface treated with poly-L-lysine
- *E. coli* cells maintained a hydrated appearance
- Smooth outer surface without surface ultrastructures or flagella resolved
- *E. coli* cells and that the bacterial cells shrunk significantly.

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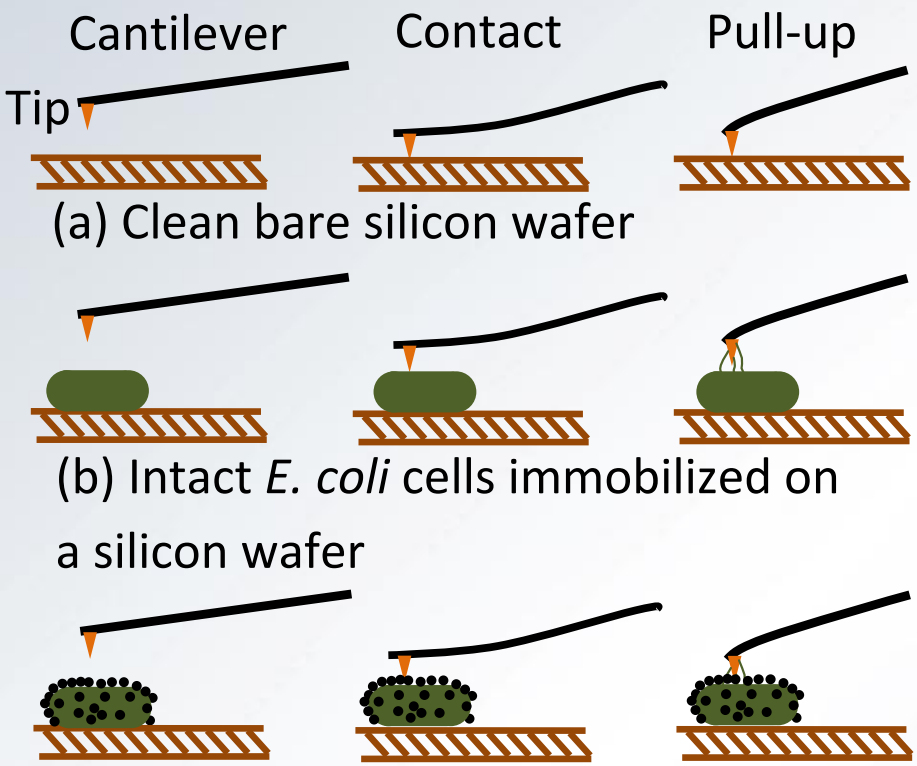
Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.

Results
2. Biomechanical property changes of *E. coli* cells at local scale



(a) Clean bare silicon wafer

(b) Intact *E. coli* cells immobilized on a silicon wafer

(c) Particle-covered *E. coli* cells immobilized on a silicon wafer (black dots represent hematite NPs)

The illustrations are not drawn to scale.

Force measurement by AFM.

- The resulting force-distance curves can be used to estimate surface hardness, elasticity, and adhesiveness.
- Hardness is indicated by the indentation of the tip engaged with the sample surface
- Elasticity is measured by spring constant of the cell
- Adhesiveness is directly reflected by the adhesion force between the tip and the cell surface

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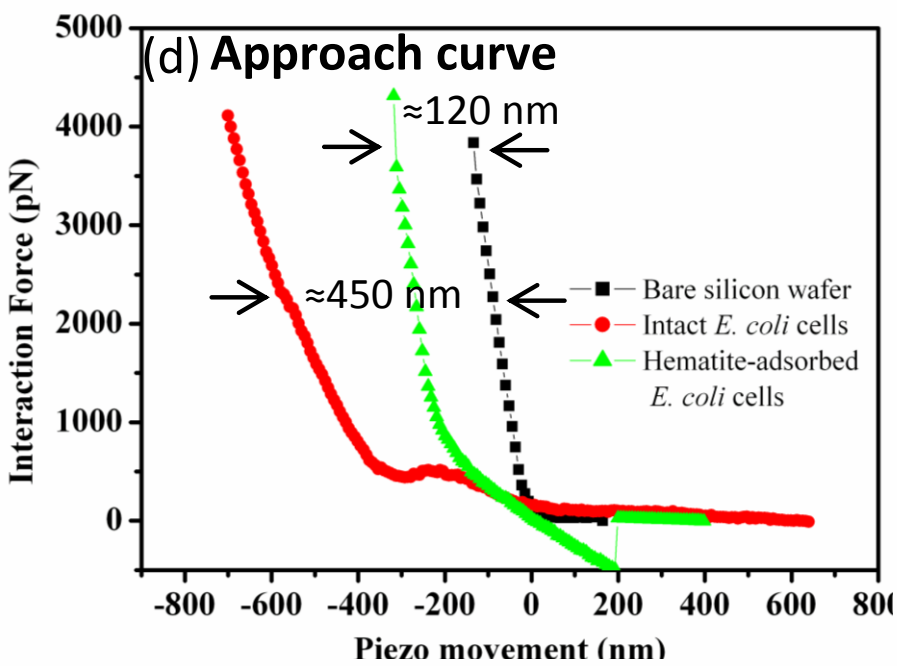

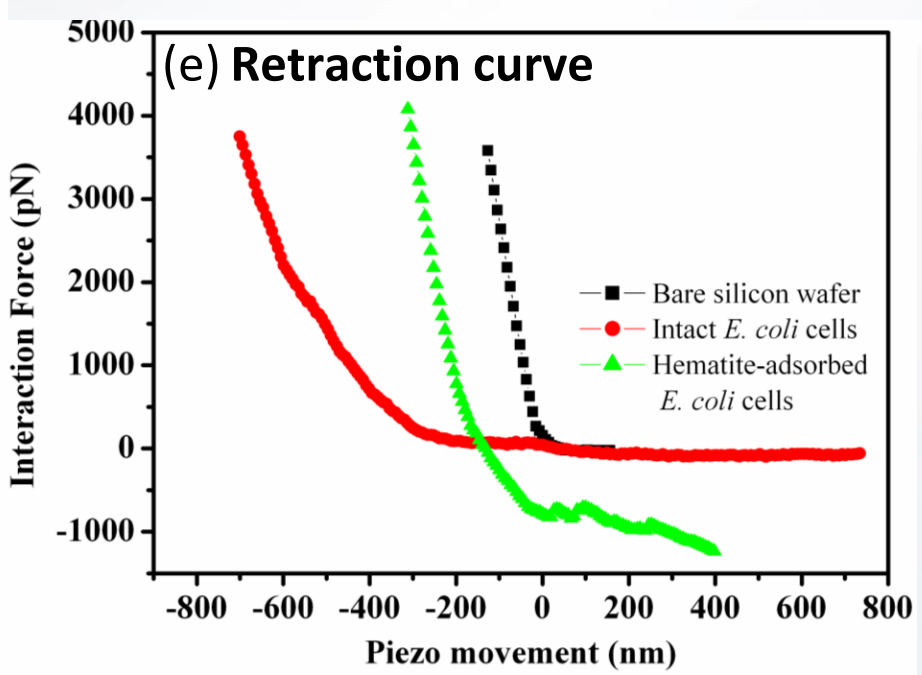

Force-distance curves as the tip approached and retracted from the contact with sample surfaces at a maximum loading force of approximately 4 nN (averaged from 6-20 replicates).

- Intact *E. coli* cells had indentation 250–450 nm, which is dependent on accurate estimation of the contact point; impaired cells only had an average indentation of 120 nm.
- No adhesion on intact untreated cells, whereas large adhesion was detected on treated cells

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.

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Results
2. Biomechanical property changes of *E. coli* cells at local scale

The spring constant of the cell (k_b) can be calculated using the equation: $k_b = k_c s / (1 - s)$

where k_c is the spring constant of the cantilever and s is the slope in the linear compliant region of the force-distance curve.

Table 1 Summary of spring constants for untreated and hematite-treated *E. coli* cells.

	Cantilever spring constant, k_c, (nN/nm)	Slope (nm/nm)*	Spring constant of the cells, k_b, (nN/nm)
Untreated *E. coli* cells	0.03±0.01	0.307 ± 0.02	0.013
Hematite-treated *E. coli* cells		0.904 ± 0.02	0.283 ➡ **Less elastic**

*The slope (nm/nm) was converted from the slope (nN/nm) in the force-distance curve dividing by the spring constant of the cantilever.

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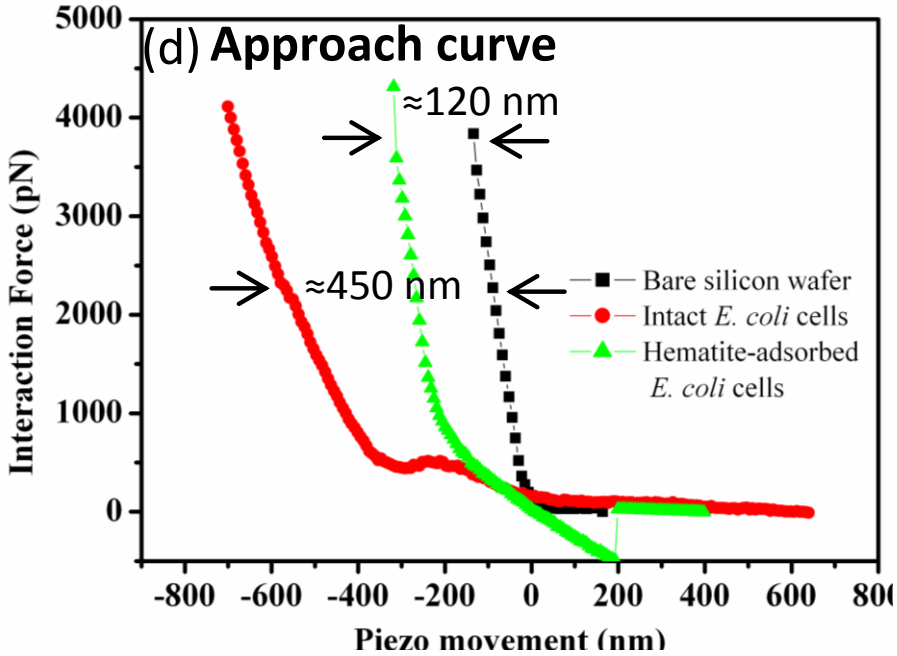
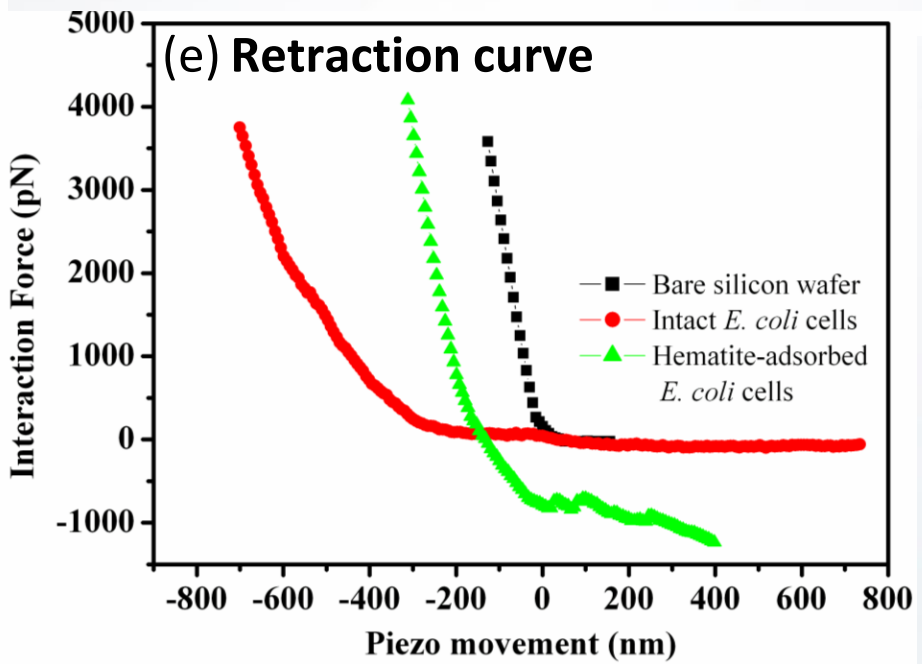
Results

2. Biomechanical property changes of *E. coli* cells at local scale





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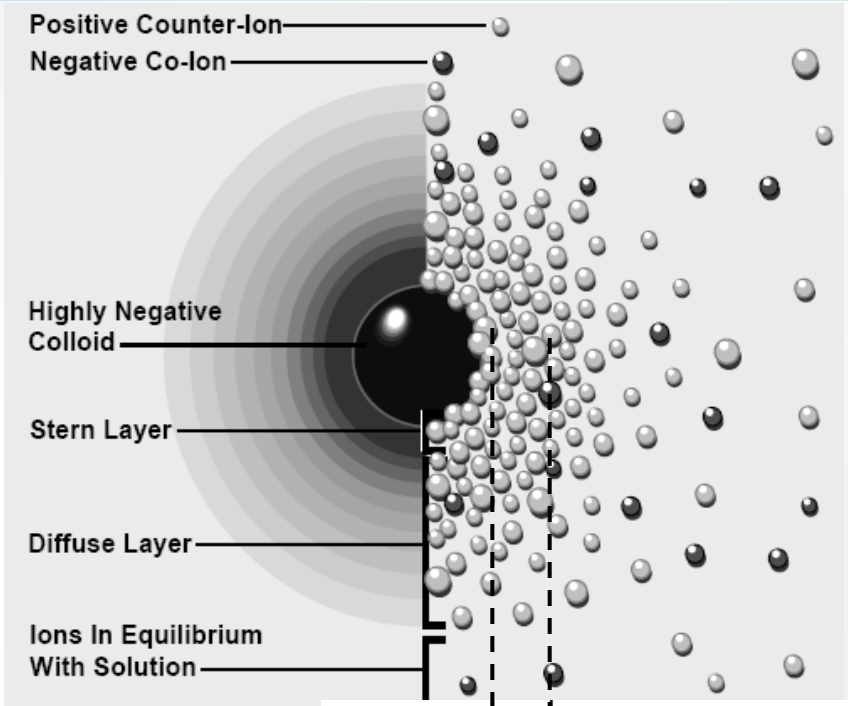
Results
3. Zeta potential and electrical double layer (EDL) theory

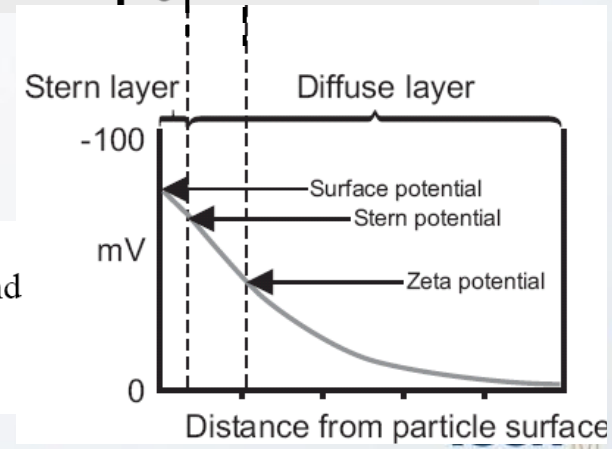
E. coli **cells, when suspended in liquid, are subject to hydration and forming a EDL. The overall electrical property can be described by zeta potential.**



Zeta potential (ζ) is commonly in aquatic chemistry and is measured by Laser Doppler electrophoresis.
The electrophoretic mobility (μ_E) is directly measured and ζ potential is converted from Henry's approximation:

$$\mu_E = \frac{2\zeta\varepsilon f(\kappa r)}{3\eta}$$

where ε is the dielectric constant (or permittivity); η is the medium's viscosity (i.e., the viscosity of water); κr is the ratio of particle radius to Debye double layer thickness; and $f(\kappa r)$ refers to Henry's function, which is 1.5 under the Smoluchowski approximation and 1 under the Hückel approximation.



See: Stumm, W.; Morgan, J. J. Aquatic chemistry. 3rd ed.; John Wiley & Sons, Inc.: New York, 1996.

Results
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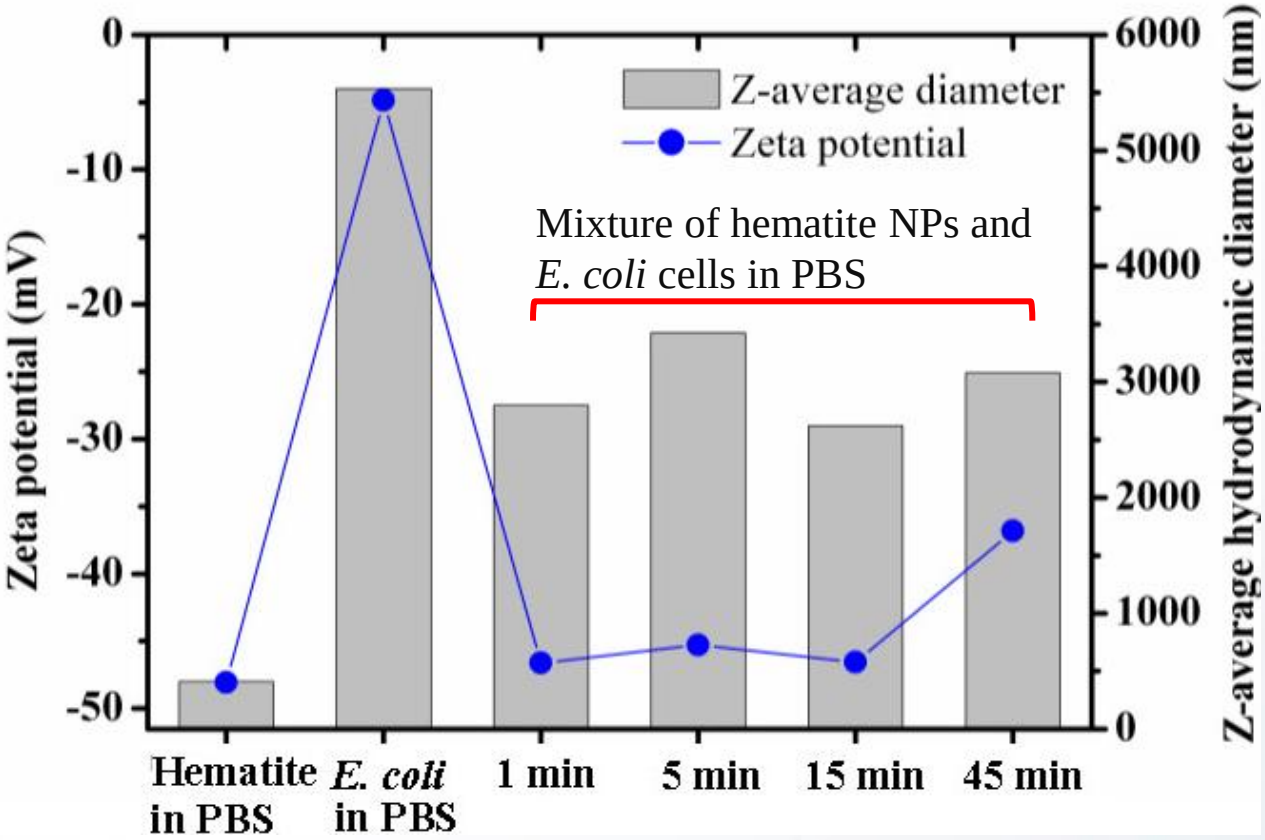

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.

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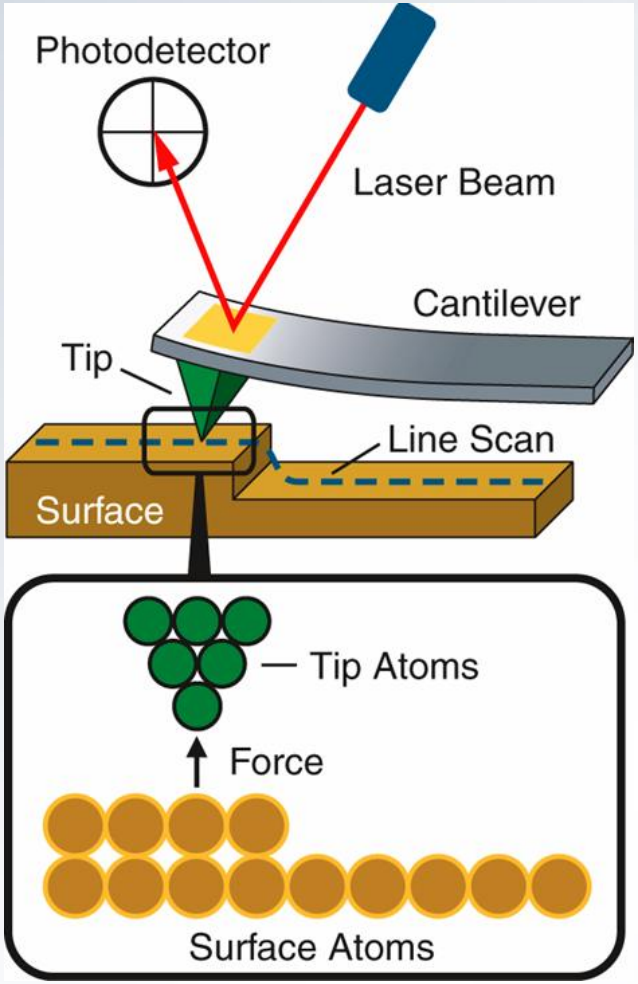
Results
4. Surface electric potential probed by KPFM



Schematic of AFM

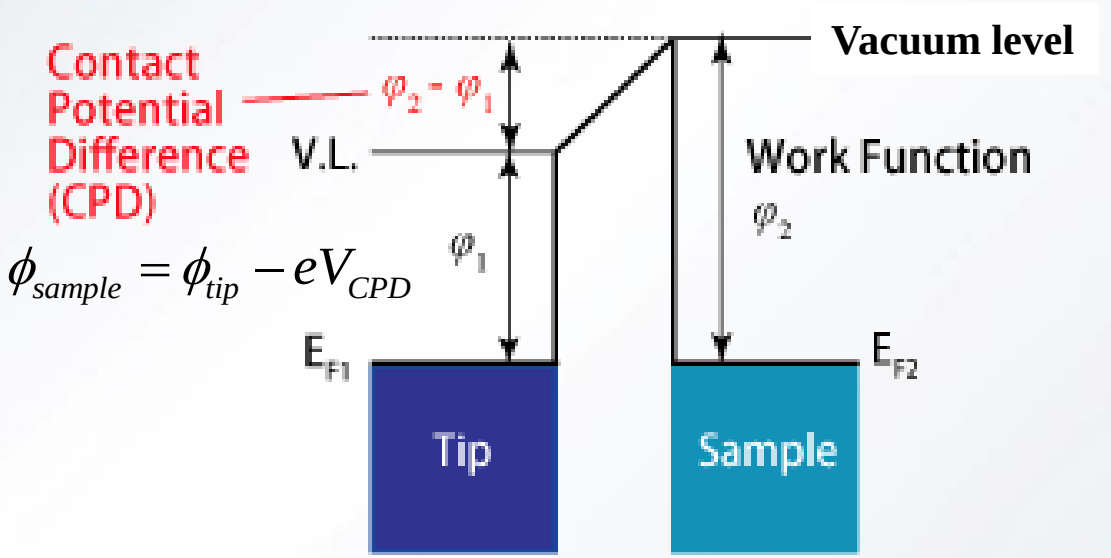
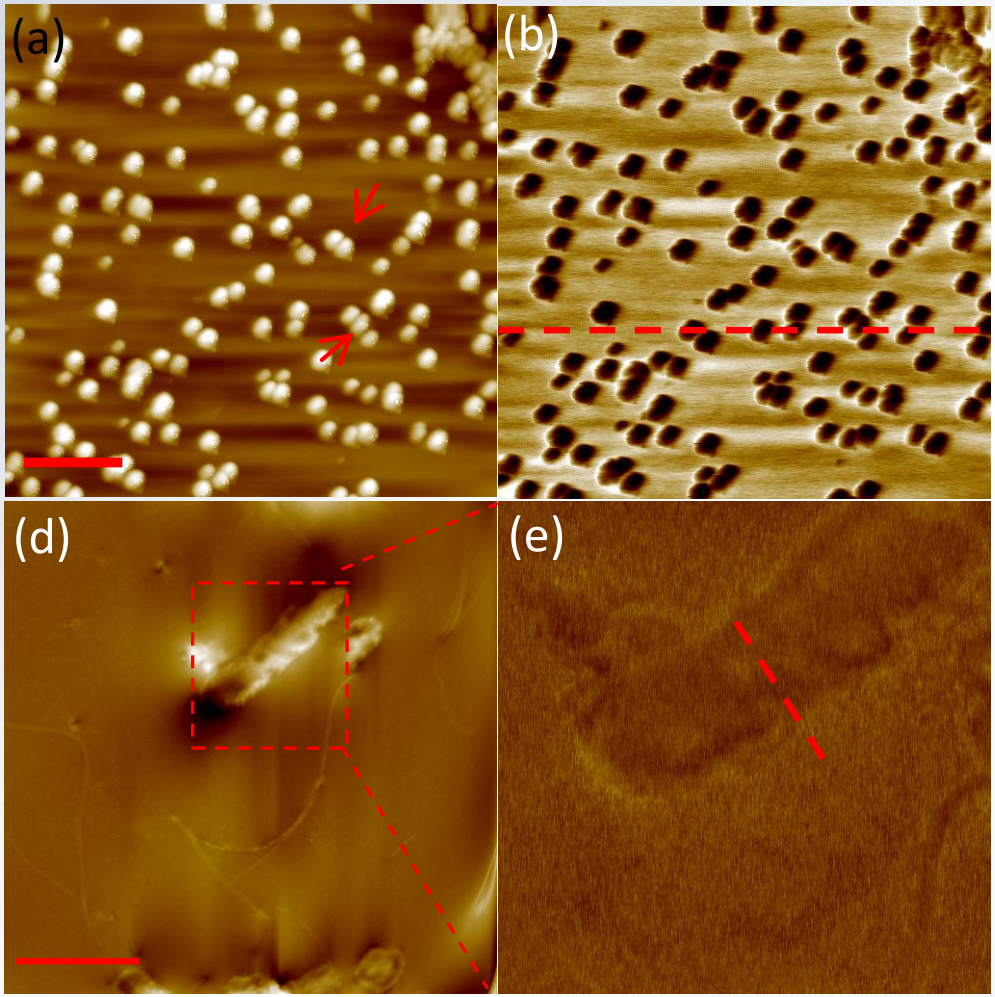
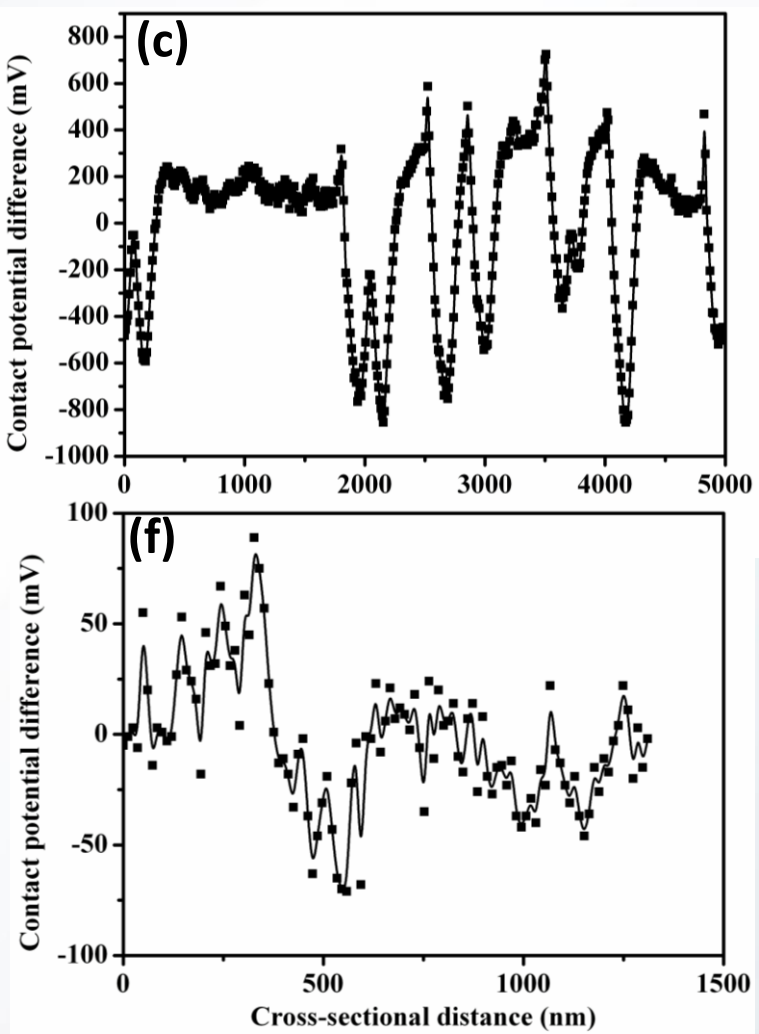


Fig. 2. Band diagram of tip and sample according to the operation principle of KPFM

In metals, **CPD can be the energy difference between the vacuum level and the Fermi energy.**
In semiconductors or biomolecules, **the work function may arise from the difference in energy between the vacuum level and the most loosely bound electron inside the sample.**

Results
4. Surface electric potential probed by KPFM





Topographical and surface potential images of hematite NPs and intact *E. coli* cells generated by KPFM. (a) and (d) are topographical images. (b) and (e) are surface potential images. (c) and (f) are cross-sectional profiles of surface potentials taken along the dashed red lines in the images in (b) and (e). The red solid lines at the bottom right in (a) and (d) indicate scale bars of 0.5 and 1 μm, respectively.

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Results
4. Surface electric potential probed by KPFM

- The relation between the work function of the conductive tip, Φ_t, and the sample, Φ_s, is given by $\Phi_s = \Phi_t - eV_{CPD}$, where e is the elementary charge and V_{CPD} is the CPD or surface potential measured by KPFM. Thus, the mean work function of hematite NPs is approximately **5.71 eV**.
- Literature reported value of the work function or Fermi level is **5.88 eV** and the minor difference is probably due to the moisture on hematite surface that induced band bending.

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.

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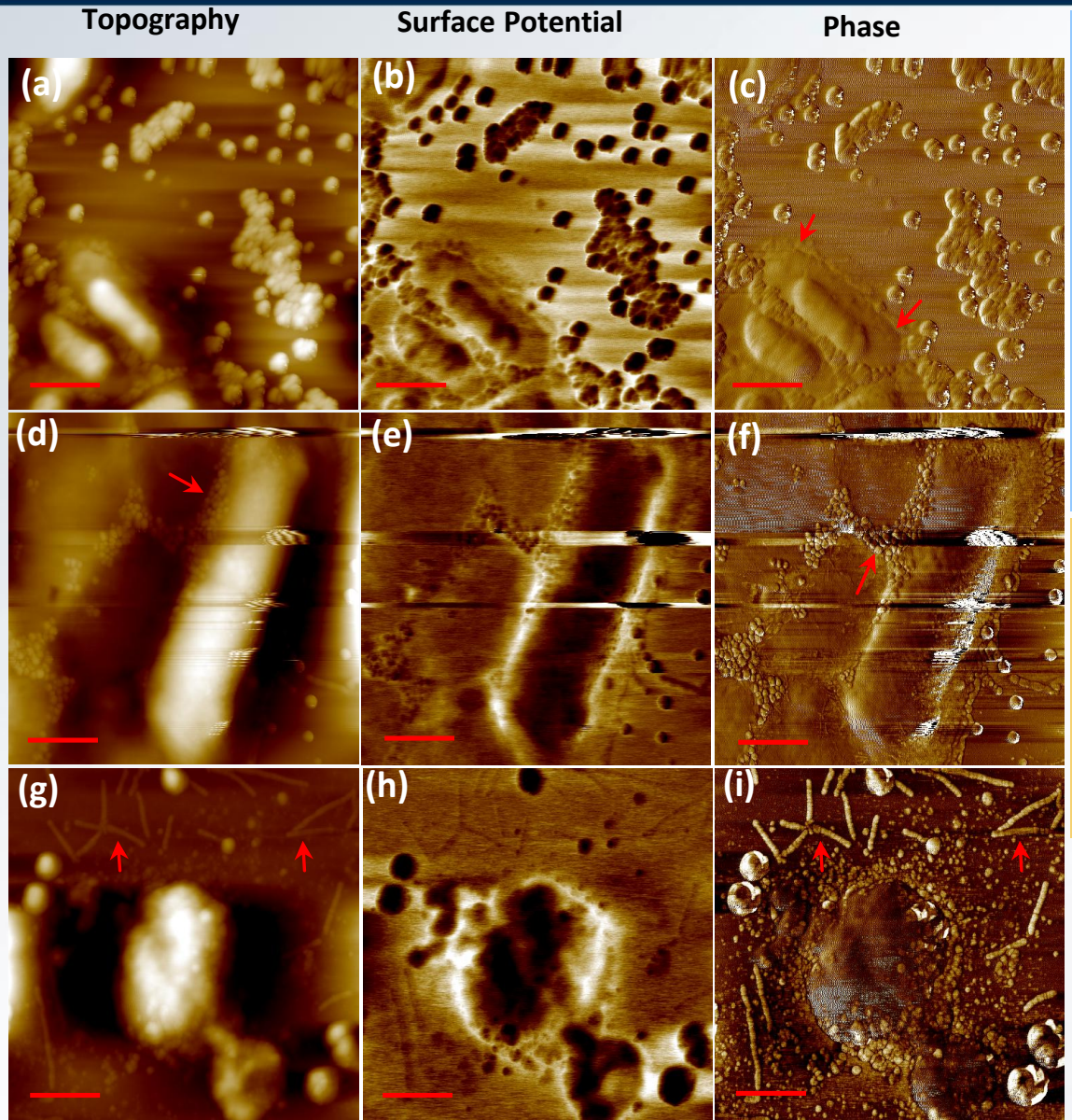
Results
4. Surface electric potential probed by KPFM



The topographical (left column), surface potential (middle column), and phase (right column) images of *E. coli* cells exposed to hematite NPs (98 nm). Exposure times were approximately (a)-(c) 3 min; (d)-(f) 10 min; and (g)-(i) 45 min. The red scale bars at the bottom right of (a)-(c) and (d)-(i) indicate lengths of 2 μm and 0.5 μm, respectively.

- Deformation observed with hematite NPs adsorbed and the increasing adsorption time
- Surface appendage (flagella) shredded and scattered around.

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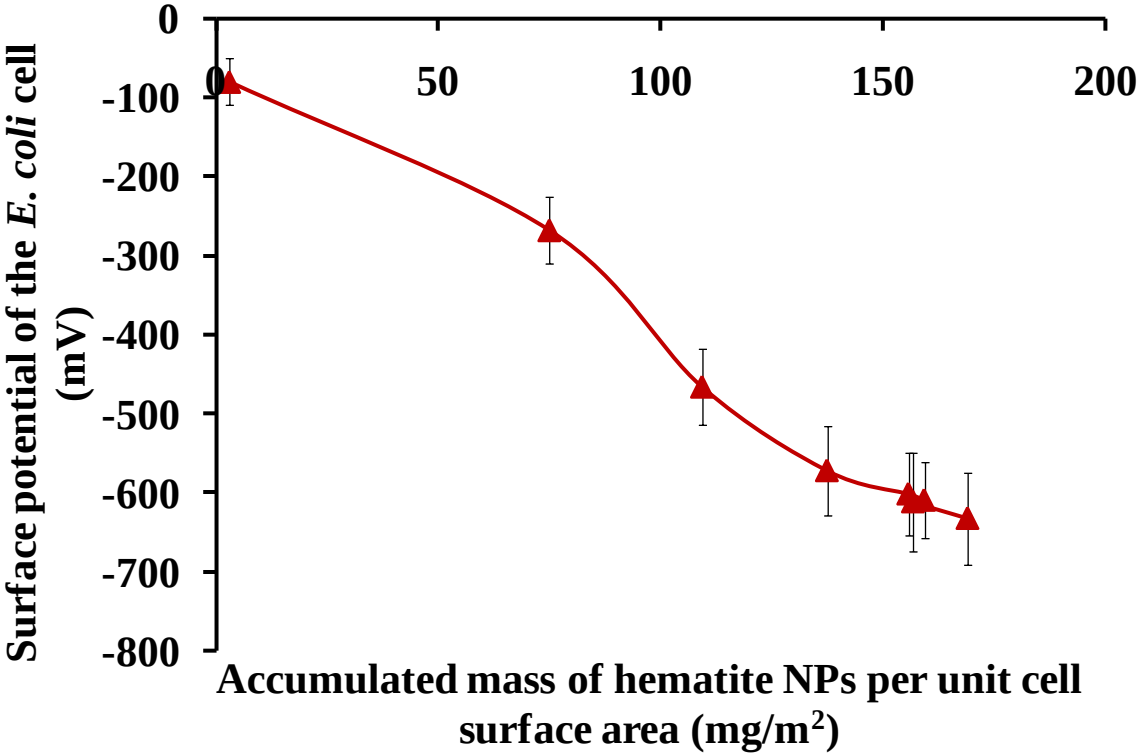
Results
4. Surface electric potential probed by KPFM



- ❑Similar to the finding of zeta potentials but……
- ❑Quite different in the meaning of the electric potential
- ❑Visualization of surface heterogeneities
- ❑Local surface potential down to the resolution of the cantilever tip diameter

Surface became more negatively charged as more hematite attached.

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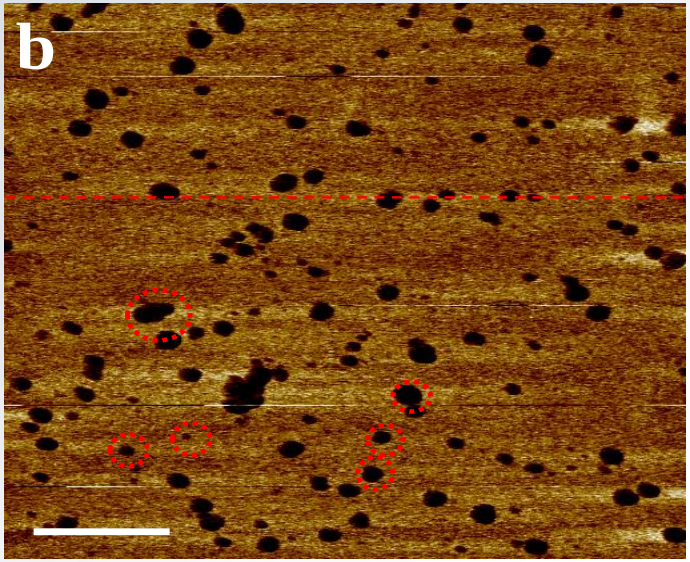
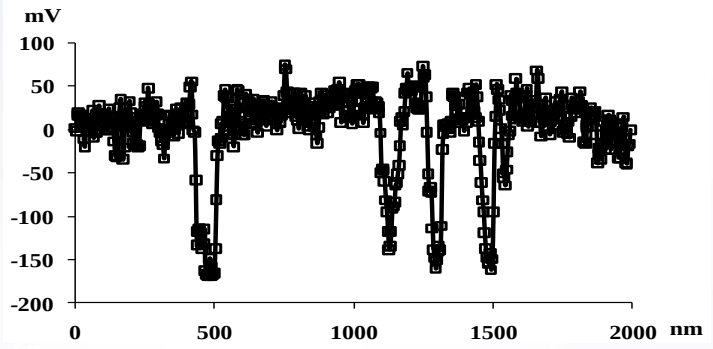
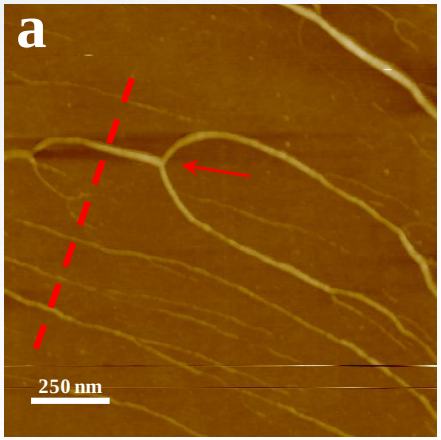
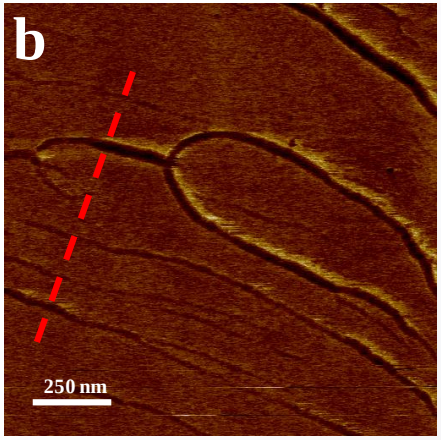
Results
4. Surface electric potential probed by KPFM

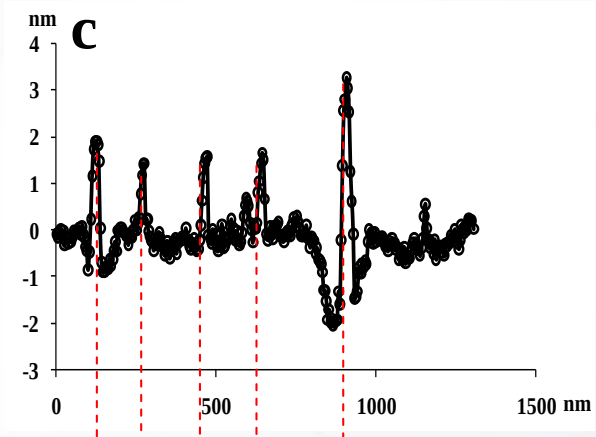
My previous work on basis of KPFM focused on differentiation of interacting entities (DNA and QDs) at nanoscale.

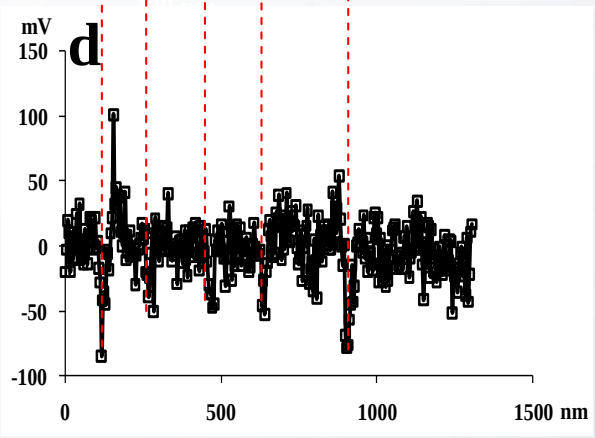












Wen Zhang, Ying Yao, and Yongsheng Chen Quantifying and Imaging the Morphology and Nanoelectric Properties of Soluble Quantum Dot Nanoparticles Interacting with DNA. ***Journal of Physical Chemistry C***, 2011, 115 (3), 599-606.

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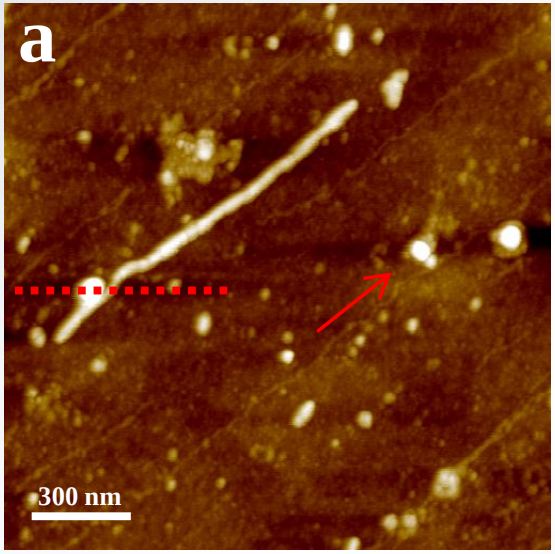
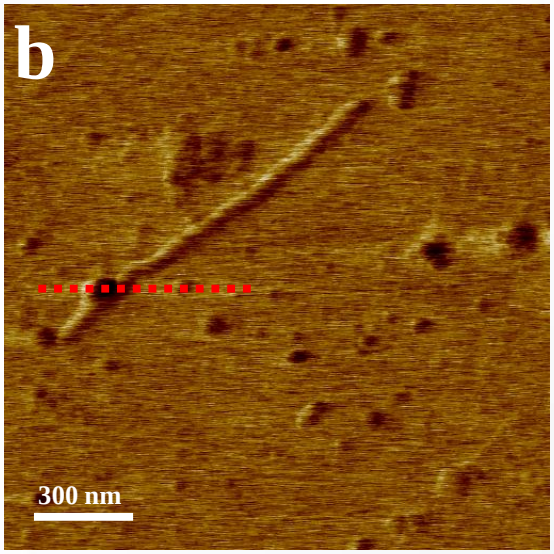
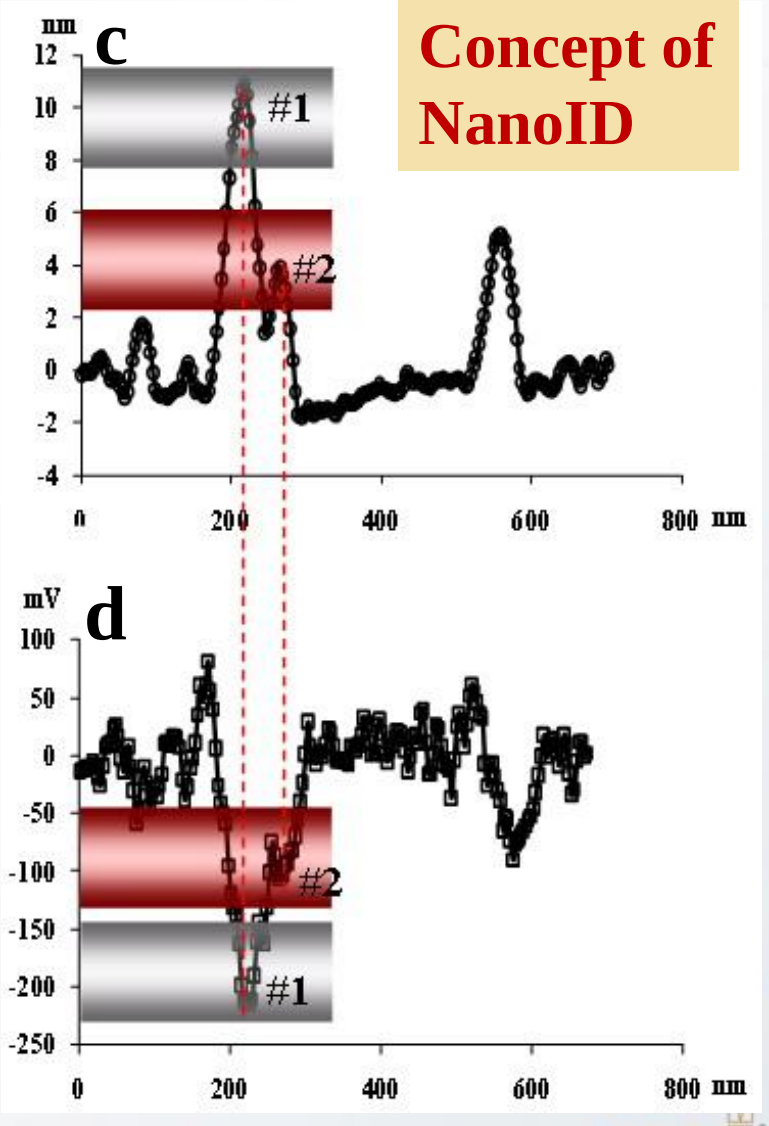
Results
4. Surface electric potential probed by KPFM

The **topography** and **surface potential** differences enable us to better differentiate and quantify QDs bound with DNA.







Concept of NanoID

Wen Zhang, Ying Yao, and Yongsheng Chen Quantifying and Imaging the Morphology and Nanoelectric Properties of Soluble Quantum Dot Nanoparticles Interacting with DNA. ***Journal of Physical Chemistry C***, 2011, 115 (3), 599-606.

Brief conclusion

- The *E. coli* cell surface became coarser, stiffer, and more adhesive with hematite NPs attached.

- Surface potential shifted to more negatively charged with the attachment of hematite NPs, observed by both zeta potential and KPFM.

Mechanisms of surface disruption from a chemical perspective

- High surface area of NPs provides more reactive sites for ROS production
- ROS formed in NP suspension usually consist of superoxide radical ($O_2^{\bullet -}$), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$)
- Representative reaction stochiometry (TiO_2 as an example):

$$TiO_2 \xrightarrow{h\nu} TiO_2(h^+_{vb} + e^-_{cb})$$
$$O_2 + e^-_{cb} \rightarrow O_2\bullet^-$$
$$O_2\bullet^- + e^-_{cb} + 2H^+ \rightarrow H_2O_2$$
$$O_2\bullet^- + H_2O_2 \rightarrow \bullet OH + OH^- + O_2$$
$$e^-_{cb} + H_2O_2 \rightarrow \bullet OH + OH^-$$
$$h^+_{vb} + OH^- \rightarrow \bullet OH$$
$$h^+_{vb} + H_2O \rightarrow H^+ \bullet OH$$
$$2\bullet OH \rightarrow H_2O_2$$

Implications:

- **Dissolution of metal ions (potentially hazardous to cells);**
- **Oxidant injury of cells, lipid peroxidation, enzyme or protein oxidation, membrane pitting, changes in membrane permeability, etc.**

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Almost all types of engineered NPs were reported to produce ROS!

NPs	DLS size in diameter (nm)	TEM Size in diameter (nm)	Results •OH	$^1O_2$	$O_2^{\bullet-}$	ROS
TiO_2	364					**Y**
	175					**N**
	175					**N**
	826-2368				√	
	800-1900				√	
	79		√	√	√	
	79				√	
			√			
						Y
	612					**N**
		15-45				**Y**
		20±3				**Y**
CeO_2	2610					**N**
		6, 12, and 1000				**Y**
		14				**Y**
ZnO	50-300	20				**Y**
	413				√	
	800					**Y**
ZnO/PEG	93					**Y**
Fe_2O_3						**Y**

NPs	DLS size in diameter (nm)	TEM Size in diameter (nm)	Results •OH	$^1O_2$	$O_2^{\bullet-}$	ROS
SiO_2-NH_4OH/NaCl/Na_3AlO_3		13.3, 15.3				**Y**
	590±104	15±5				**Y**
Si- NH_2		1.6±0.2 (only core)				**Y**
Si- N_3		1.6±0.2 (only core)				**Y**
Si- COOH		1.6±0.2 (only core)				**N**
Ag		15, 100	√			
		5-10				**Y**
		15				**Y**
Ag- PVA		15				**Y**
		15				**Y**
γ-Al_2O_3		50	√			
α-Al_2O_3		300	√			
AuNPs-*TPPMS*		1.4				**Y**
AuNPs	22.1±1.9					**Y**
Co_3O_4		11.4 (by SSA)				**Y**

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Almost all types of engineered NPs were reported to produce ROS!

NPs	DLS size in diameter (nm)	TEM Size in diameter (nm)	Results			
			•OH	$^1O_2$	$O_2^{\bullet-}$	ROS
Mn_3O_4		12.2 (by SSA)				**Y**
$_2O_3$		12	√			
CdTe QDs					√	
InP/ZnS QDs		2.8	√		√	
CdS QDs-mercaptoacetic acid (MPA)		3	√		√	
CdSe QDs-MPA)		4.4	√			
CdSe/ZnS QDs- MPA)		4				**N**
CdSe/ZnS QDs- biotin			√		√	
CdTe QDs - MPA	20			√		
CdTe QDs-MPA				√		
Polystyrene-NH_2	65	<100				**N**
Polystyrene-NH_2	527	<100				**Y**
Polystyrene-COOH	56	<100				**N**
	82	<100				**N**
Fullerene	20.5			√		
			√		√	
Fullerene-PVP				√		
	4.4			√	√	
						Y
Fullerene-polymer (polyvinylprrolidone)				√		
Fullerene-Triton X100; Brij 78; Ozonated C_{60}				√	√	
			√	√	√	
Aggregates of C_{60}	64, 84					**N**
	122			√		
	122			√	√	
fullerol(hydroxylated C_{60})				√		
	218					**Y**
	106					**N**

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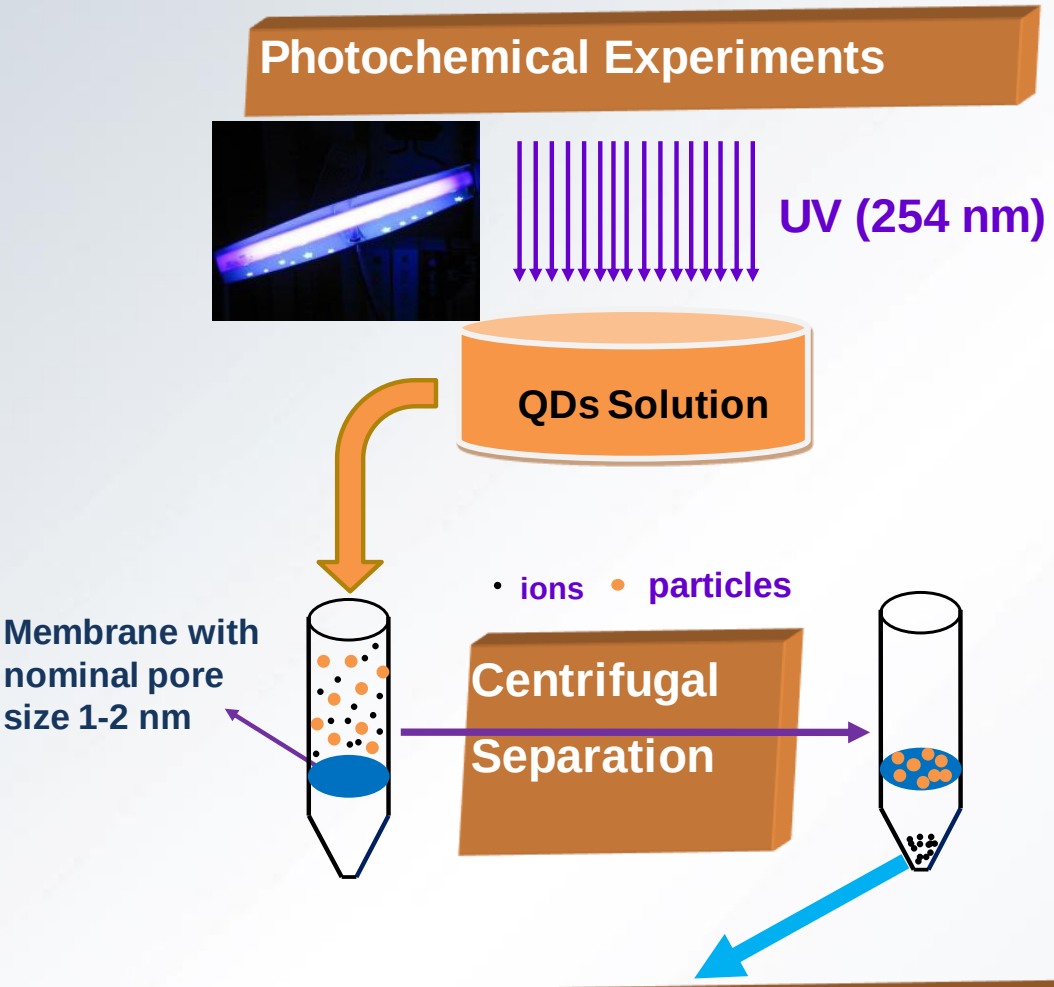
Example 1: Photoleaching or photo-oxidation of QDs, a chemical reaction driven by ROS production



- Environmental effects (irradiation intensity, temperature, dissolved oxygen, and dissolved organic matter)
- Coating effect

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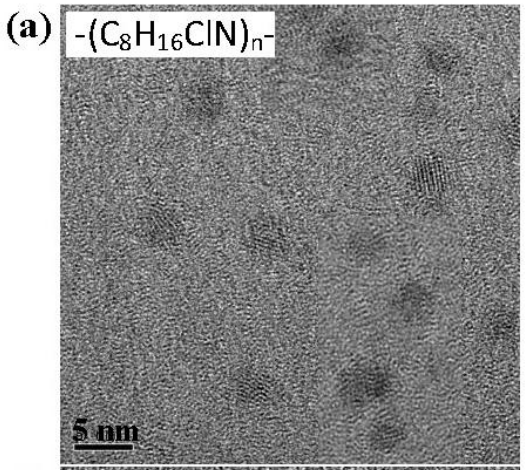
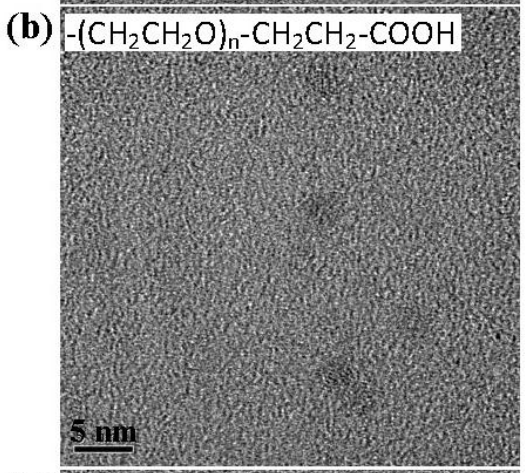
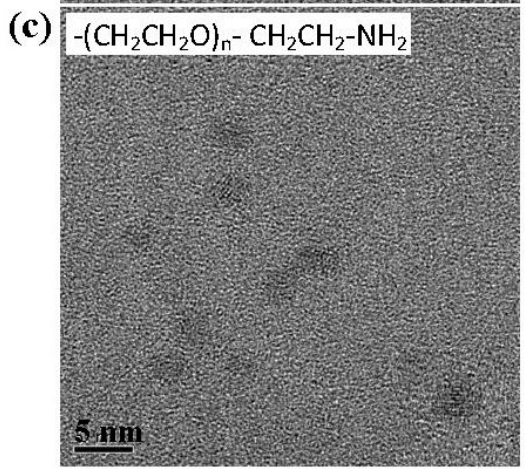
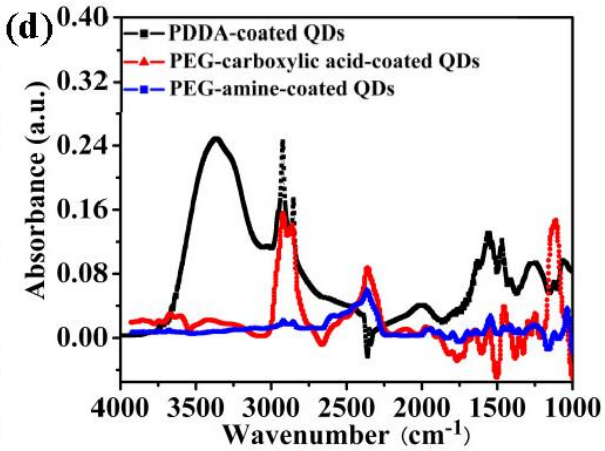
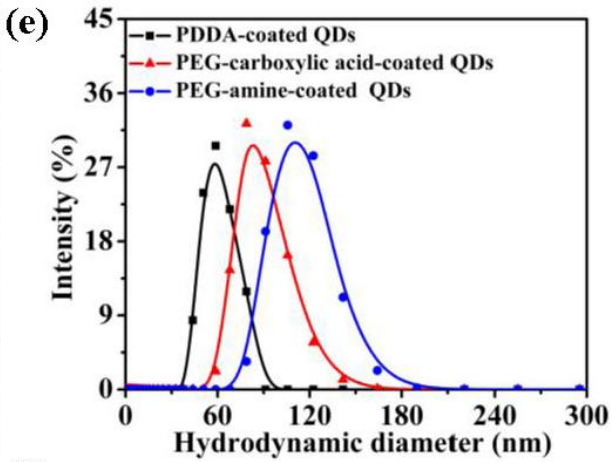
Characterization of QDs

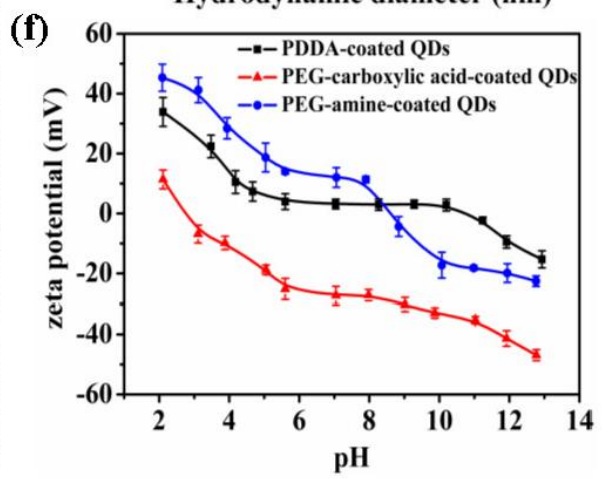












(a)-(c) HR-TEM images of Polydiallydimethylammounium chloride (PDDA)-, PEG-carboxylic acid-, and PEG-amine-coated QDs, (d) FTIR spectra, (e) Intensity-averaged PSD diagrams, and (f) zeta-potentials as a function of pH for the three types of QDs.

1. **CdSe/ZnS as the core**
2. **3-5 nm in diameter (TEM)**
3. **Different surface coating and surface charge**

Yang Li, Wen Zhang, Kungang Li, Ying Yao, Junfeng Niu, and Yongsheng Chen. Oxidative dissolution of polymer-encapsulated CdSe/ZnS quantum dots under UV irradiation: mechanisms and kinetics. *Journal of Physical Chemistry C*, Submitted.

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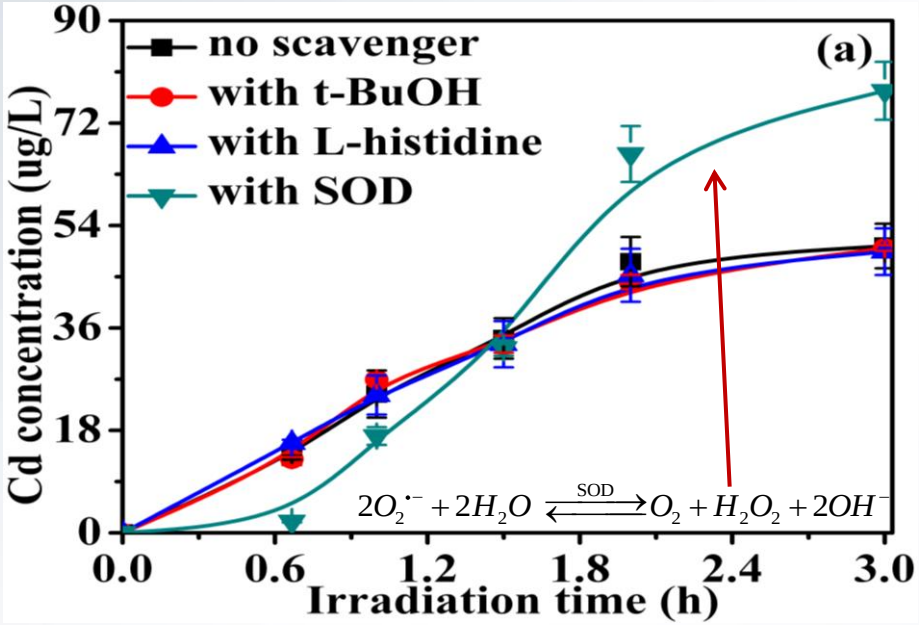
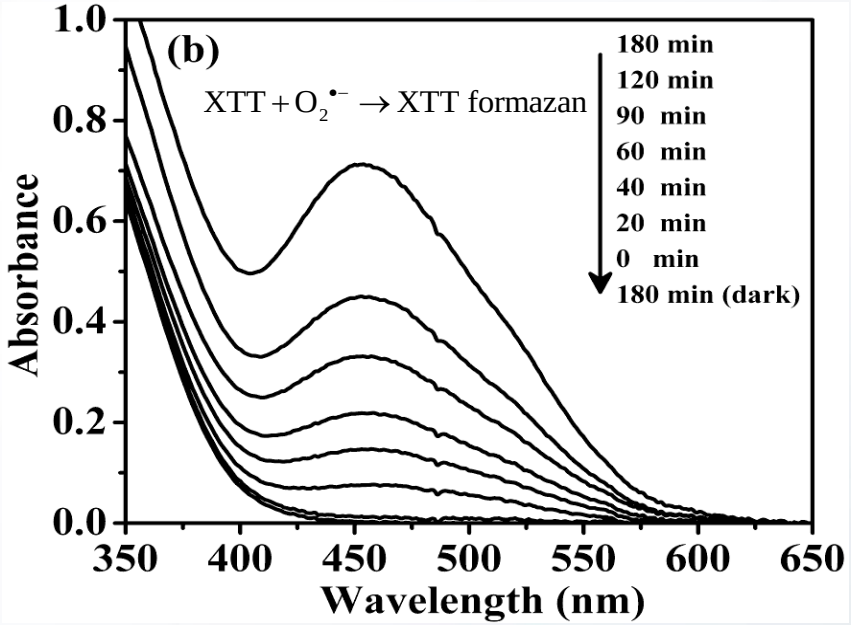
Example 1: ROS measurement, an indirect indication using scavengers

Scavenging experiments:

- **t-BuOH (30 mM), l-histidine (80 mM) and superoxide dismutase (SOD) (2000 unit/L) from *Escherichia coli* were used to scavenge •OH, $^1O_2$, and $O_2^{\bullet-}$, respectively.**
- **Monitoring the release rates of Cd and Se to indicate the presence of ROS ($O_2^{\bullet-}$).**





(a) Dissolution kinetics of PDDA-coated QDs in the presence and absence of scavengers (initial concentration of QDs 140±3 μg-Cd/L, t-BuOH 30 mM, l-histidine 80 mM, and SOD 2,000 unit/L), (b) UV-Vis absorption spectra of PDDA-coated QDs in the presence of XTT as a function of irradiation time (initial concentration of QDs 1.4±0.03 mg -Cd/L and XTT 0.15 mM)

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Yang Li, Wen Zhang, Kungang Li, Ying Yao, Junfeng Niu, and Yongsheng Chen. Oxidative dissolution of polymer-encapsulated CdSe/ZnS quantum dots under UV irradiation: mechanisms and kinetics. *Journal of Physical Chemistry C*, Submitted.

Example 2: Another indirect ROS measurement method using indicators

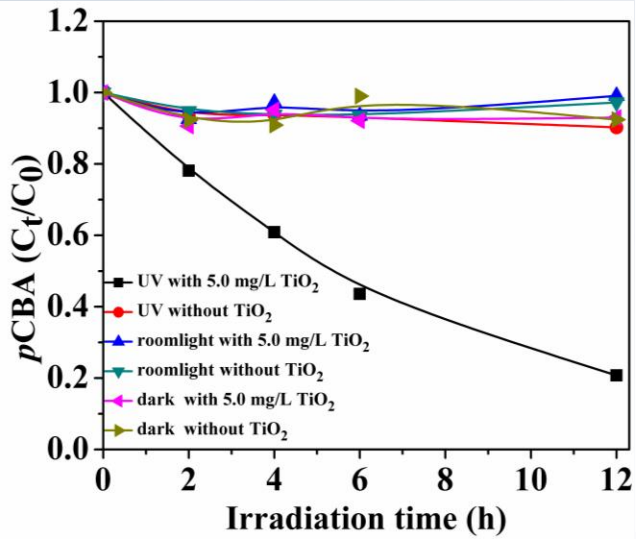
Methods of probing ROS generation from different types of NPs

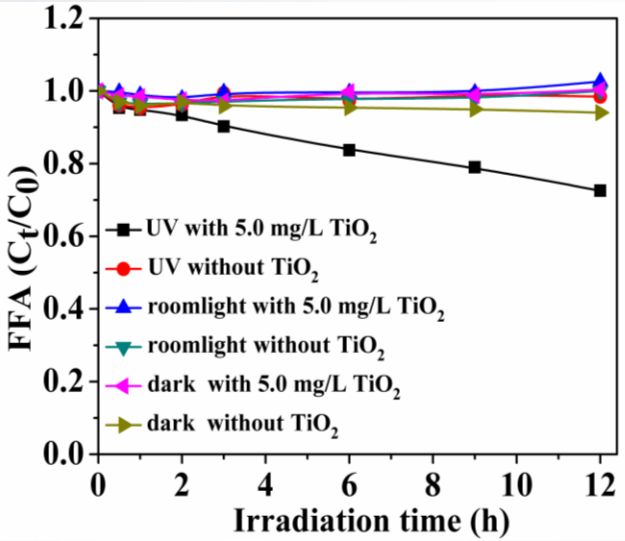
ROS	•OH	$^1O_2$	$O_2^{\bullet-}$
Method	HPLC	HPLC	UV-Vis (430 nm)
Indicator	*p*CBA	FFA	XTT

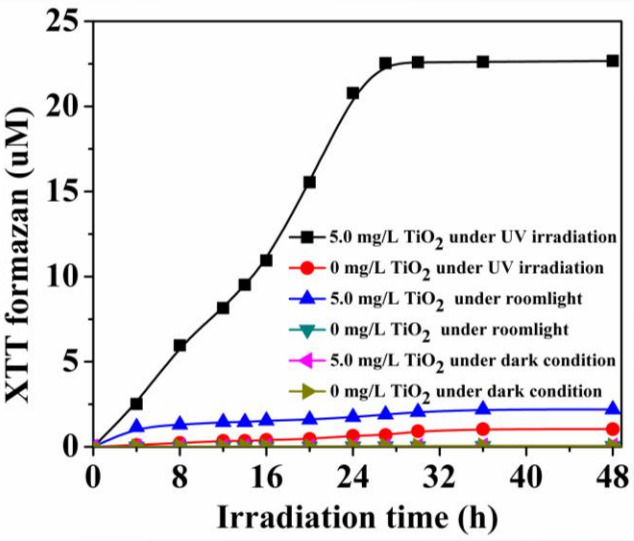
Preliminary results

NPs	•OH	$^1O_2$	$O_2^{\bullet-}$
TiO_2	√	√	√
CeO_2			√
SiO_2			
ZnO	√		√
CuO	Under investigation		
AuNPs			

ROS generation on different NPs (TiO_2, CeO_2, and ZnO as examples)







- TiO_2 NPs produces three types of ROS under UV.
- Under room light or dark environment, no significant ROS were detected.

Yang Li, Wen Zhang, Junfeng Niu, and Yongsheng Chen. Reactive oxidative species generation from engineered nanoparticles: kinetics and toxicity implications. *In preparation*.

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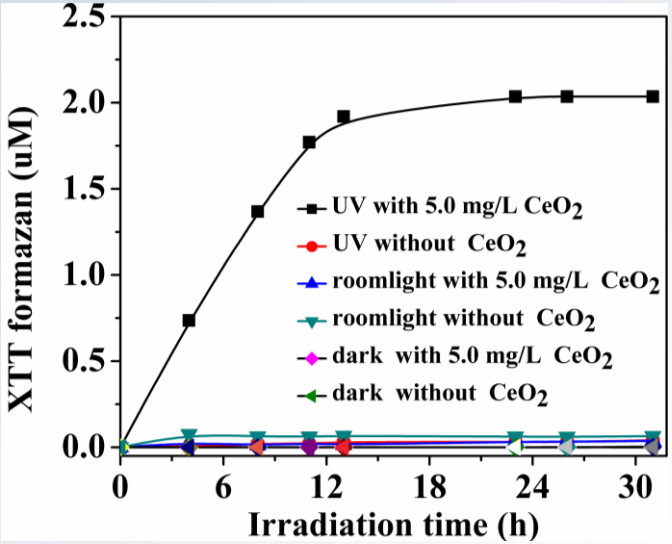
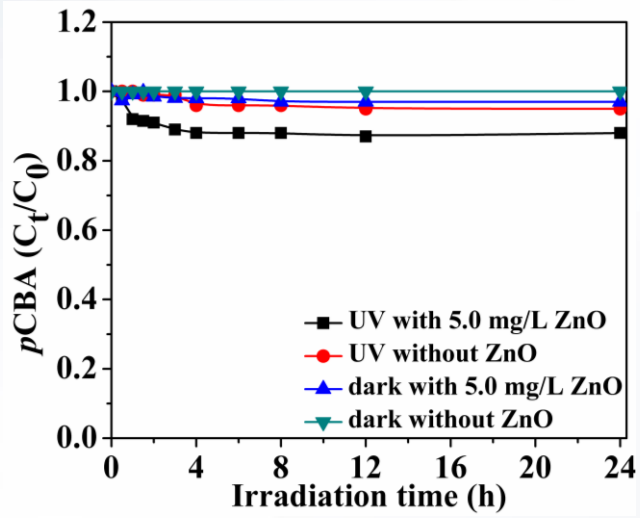
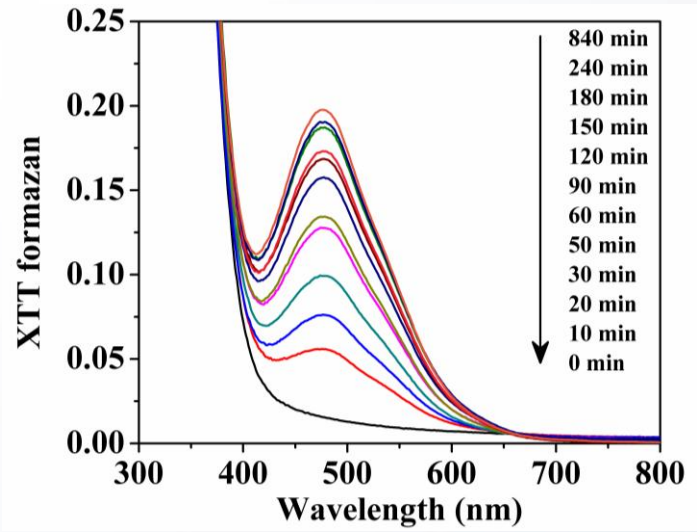
ROS generation on different NPs (TiO_2, CeO_2, and ZnO as examples)



➢CeO2 NPs were found to produce $O_2^{\bullet-}$ only.



➢ZnO NPs produced •OH and $O_2^{\bullet-}$



Yang Li, Wen Zhang, Junfeng Niu, and Yongsheng Chen. Reactive oxidative species generation from engineered nanoparticles: kinetics and toxicity implications. *In preparation*.

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Quantitative relationship between ROS production and toxic potential (ongoing research)

Now, I propose to assign the weight factors and calculate the "ROS index" for each type of NPs that is further used to draw a relationship with mortality rates (as an indicator of toxic potential).

Different ROS/NPs	•OH	$^1O_2$	$O_2^{\bullet-}$
Weight	A1	A2	A3
ROS production rate constant (s^{-1})	k1	k2	k3
ROS index=	A1·k1+A2·k2+A3·k3		

Yang Li, Wen Zhang, Junfeng Niu, and Yongsheng Chen. Reactive oxidative species generation from engineered nanoparticles: kinetics and toxicity implications. *In preparation*.

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Take-home messages

- Physical and chemical insight into nanotoxicity.
- Changes in bioimechanical and electrical properties may interpret the surface disruption
- ROS leads to the chemical irritation for cells and is widely detected in many types of engineered NPs.

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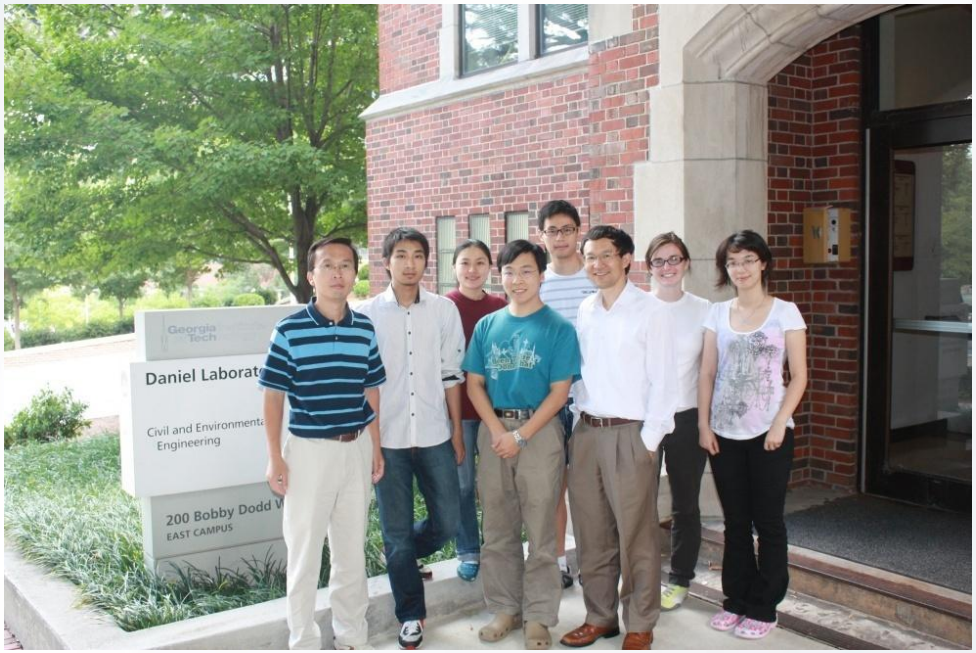

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