Development of Quantitative Structure-Activity Relationship for Prediction of Biological Effects of Nanoparticles Associated with Semiconductor Industries

(*Task Number: 425.025*)

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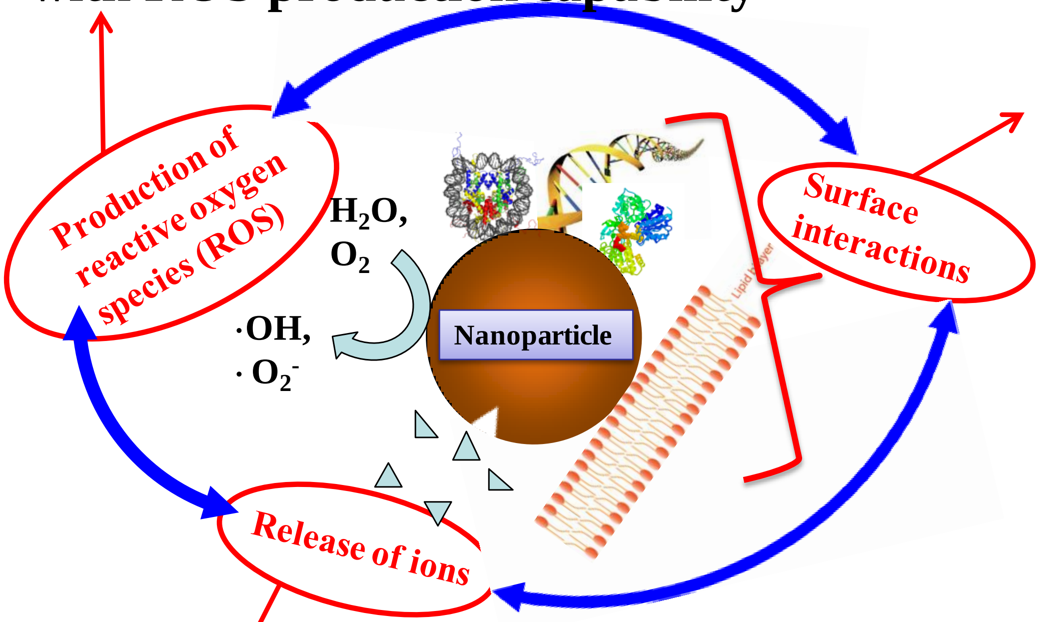
Cost Share (other than core ERC funding):

- $400k DOE-BES
- $300k NSF CBET
- $60 k National Academic of Science Ford Fellowship to Charlie Corredor
- $25 k start-up fund from ASU
- $60k start-up for consumables and
- $152k funds from GIT for AFM and other lab instrument purchase

Method and Approach

- **Perform study on ROS production**
- **Explore ROS-production mechanism with band gap theory**
- **Correlate the NP antibacterial activity with ROS production capability**



- **Investigate the ion release (e.g. Ag NPs)**
- **Model ion release kinetics on the basis of hard sphere collision theory**

- **Perform study on aggregation kinetics of NPs**
- **Model the aggregation of NPs with extended Derjaguin–Landau–Verwey–Overbeek (EDLVO) theory**

- **Investigate the toxicity of NPs to *Paramecium***
- **Correlate the NP toxicity with NP-cell surface interactions**

- **Use model cell membranes to study the NP adsorption and membrane disruption, as predictor of bioaccumulation and toxicity**

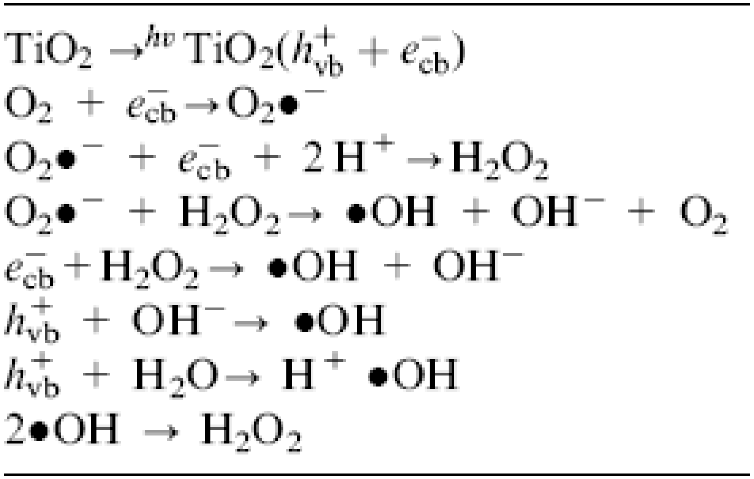
Wen Zhang, Ying Yao, Nicole Sullivan, and Yongsheng Chen. *Environ. Sci. Technol*. 2011
Stephen Klaine, et al. *Environ. Toxicol. Chem*. 2008

Highlight of Results

1. **ROS production by NPs and correlation with their antibacterial activity**
2. **Aggregation kinetics of NPs in aqueous solution;**
3. **Acute toxicity of engineered metal oxide NPs to *paramecium***
4. **Using model cell membranes as predictor of bioaccumulation and toxicity**

1.1. Cytotoxic implication of ROS production by NPs

- 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):



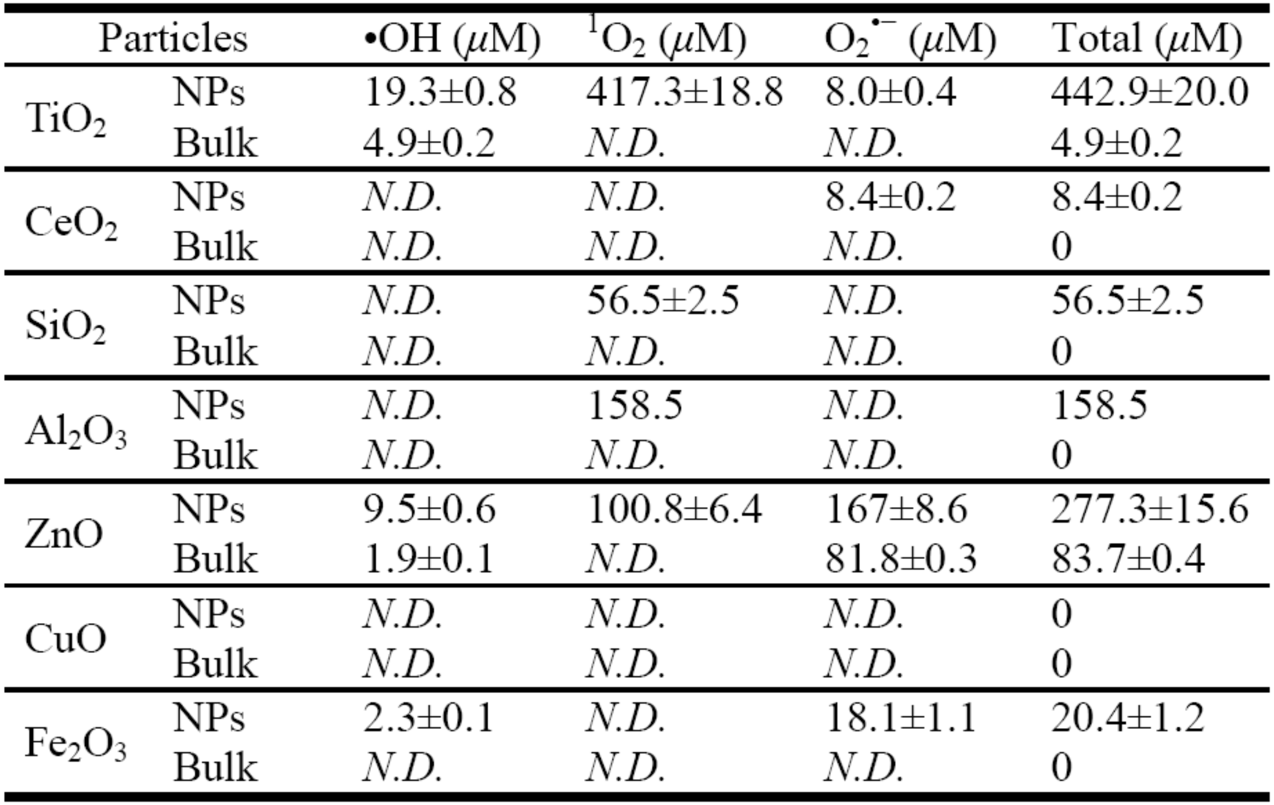
$$TiO_2 \xrightarrow{h\nu} TiO_2(h_{vb}^{+} + e_{cb}^{-})$$
$$O_2 + e_{cb}^{-} \rightarrow O_2\bullet^{-}$$
$$O_2\bullet^{-} + e_{cb}^{-} + 2\,H^{+} \rightarrow H_2O_2$$
$$O_2\bullet^{-} + H_2O_2 \rightarrow \bullet OH + OH^{-} + O_2$$
$$e_{cb}^{-} + H_2O_2 \rightarrow \bullet OH + OH^{-}$$
$$h_{vb}^{+}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

Implications:

Oxidant injury of cells, lipid peroxidation, enzyme or protein oxidation, membrane pitting, changes in membrane permeability, etc.

1.2. ROS measurement results with indicator method

Table 1. Concentrations of ROS generated by different metal oxides under UV irradiation.



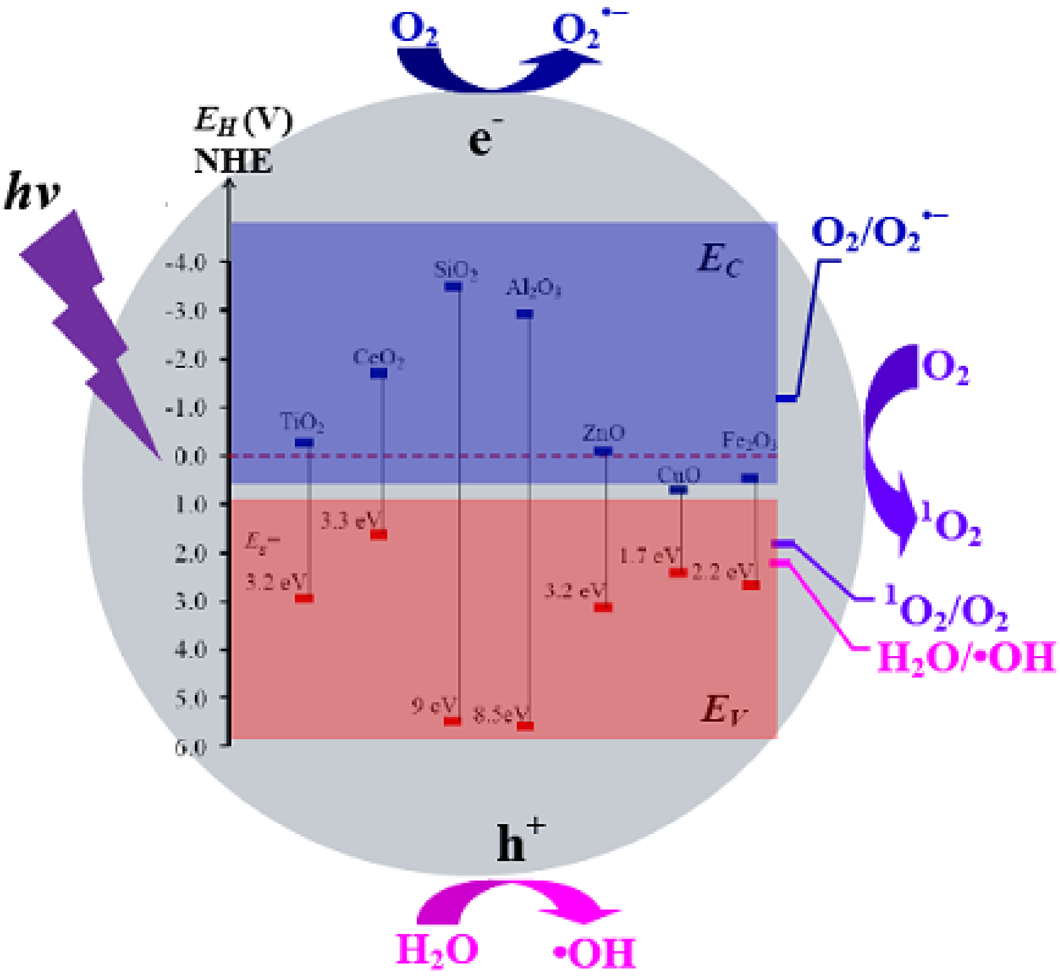
Particles		•OH (μM)	$^1O_2$ (μM)	$O_2^{\bullet -}$ (μM)	Total (μM)
TiO_2	NPs	19.3±0.8	417.3±18.8	8.0±0.4	442.9±20.0
	Bulk	4.9±0.2	*N.D.*	*N.D.*	4.9±0.2
CeO_2	NPs	*N.D.*	*N.D.*	8.4±0.2	8.4±0.2
	Bulk	*N.D.*	*N.D.*	*N.D.*	0
SiO_2	NPs	*N.D.*	56.5±2.5	*N.D.*	56.5±2.5
	Bulk	*N.D.*	*N.D.*	*N.D.*	0
Al_2O_3	NPs	*N.D.*	158.5	*N.D.*	158.5
	Bulk	*N.D.*	*N.D.*	*N.D.*	0
ZnO	NPs	9.5±0.6	100.8±6.4	167±8.6	277.3±15.6
	Bulk	1.9±0.1	*N.D.*	81.8±0.3	83.7±0.4
CuO	NPs	*N.D.*	*N.D.*	*N.D.*	0
	Bulk	*N.D.*	*N.D.*	*N.D.*	0
Fe_2O_3	NPs	2.3±0.1	*N.D.*	18.1±1.1	20.4±1.2
	Bulk	*N.D.*	*N.D.*	*N.D.*	0

N.D. **indicates that ROS were not detected or were not statistically significant.**

- **Al_2O_3 NPs were found to produce $^1O_2$ only.**
- **CeO_2 NPs were found to produce $O_2^{\bullet -}$ only.**

Yang Li, Wen Zhang, Junfeng Niu, and Yongsheng Chen. Mechanism of Photogenerated Reactive Oxygen Species and Correlation with Antibacterial Properties of Engineered Metal Oxide Nanoparticles. *ACS Nano*. Submitted

1.3. ROS production mechanism



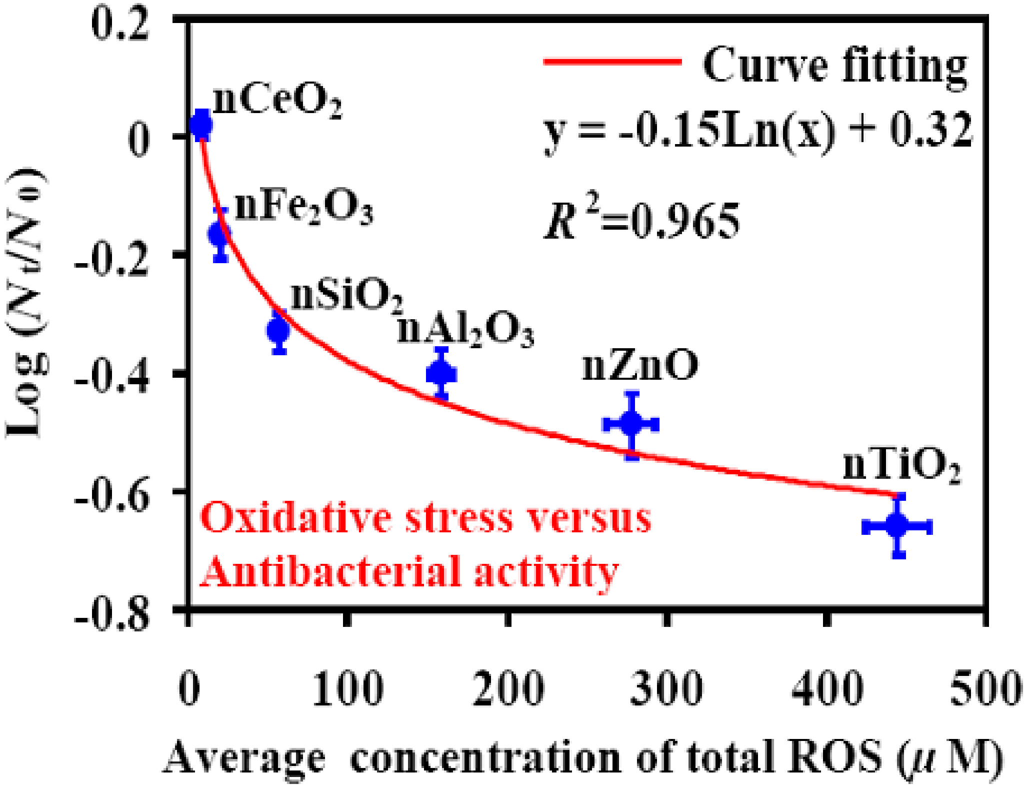
- **Electrons jumping from the valence band to the conduction band will form holes and free electrons.**

- **The hole and free electron have strong oxidizing and reducing power, respectively.**

- **Compare the band edge energy levels of the metal oxides with the redox potentials.**

Grätzel, M. *Nature* 2001, 414.
Yang Li, Wen Zhang, Junfeng Niu, and Yongsheng Chen. Mechanism of Photogenerated Reactive Oxygen Species and Correlation with Antibacterial Properties of Engineered Metal Oxide Nanoparticles *ACS Nano*. Submitted

1.4. ROS production correlates with antibacterial activity of NPs

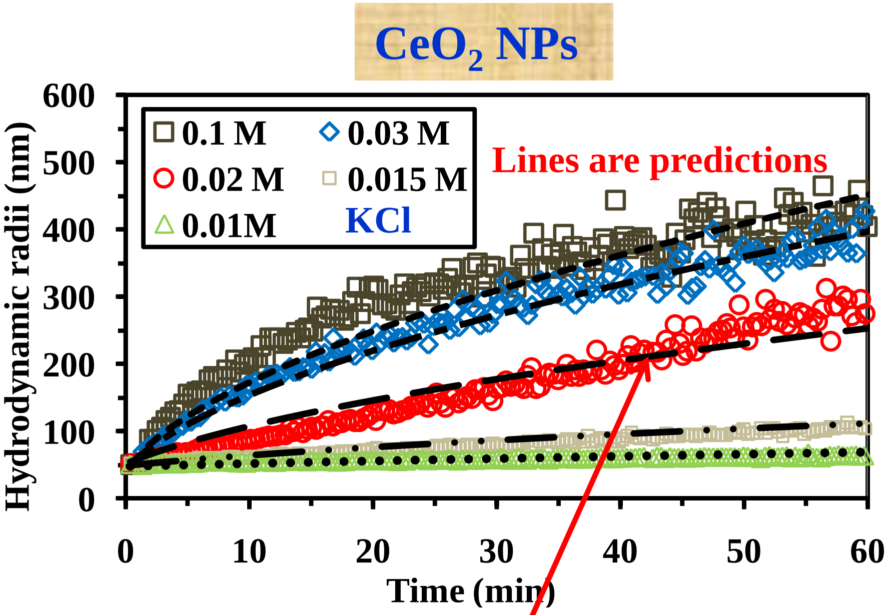


Total ROS=$[O_2^{\bullet -}]+[\bullet OH]+[^1O_2]$

NPs which produce higher level ROS killed more *E.coli* cells

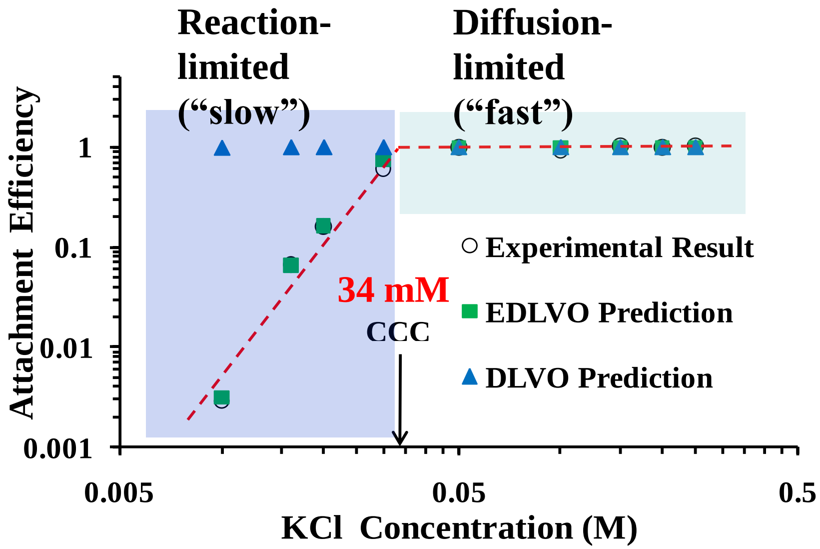
Yang Li, Wen Zhang, Junfeng Niu, and Yongsheng Chen. Mechanism of Photogenerated Reactive Oxygen Species and Correlation with Antibacterial Properties of Engineered Metal Oxide Nanoparticles *ACS Nano*. Submitted

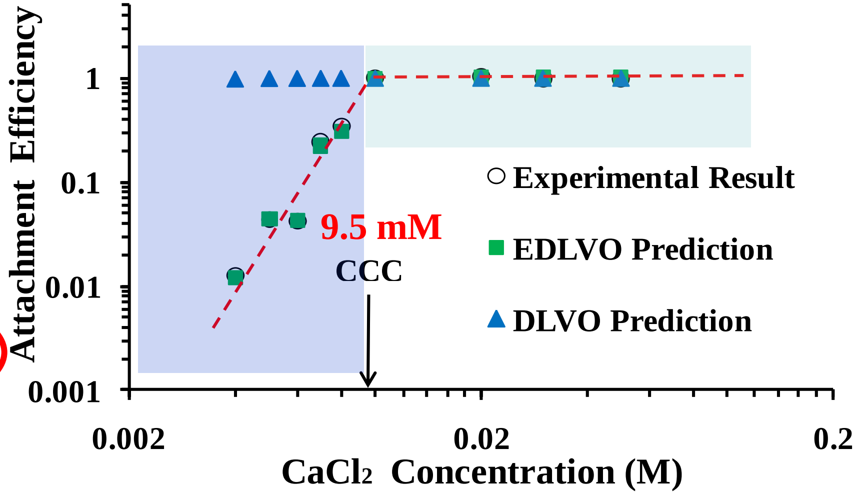
2.1. Ionic strength effect on aggregation kinetics of NPs



$$r = a \cdot \left\{ 1 + \frac{4k_B T n_0}{3\mu W} t \right\}^{1/d_F}$$

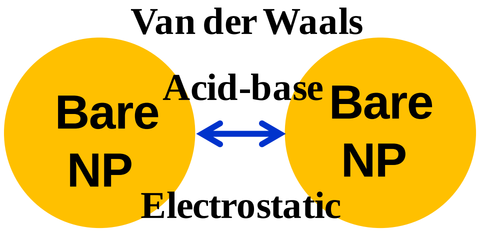
$$W = \left[\int_0^\infty \lambda(u) \frac{\exp\left(V_T(u)/kT\right)}{(2+u)^2} du \right] \cdot \left[\int_0^\infty \lambda(u) \frac{\exp\left(V_A(u)/kT\right)}{(2+u)^2} du \right]^{-1}$$

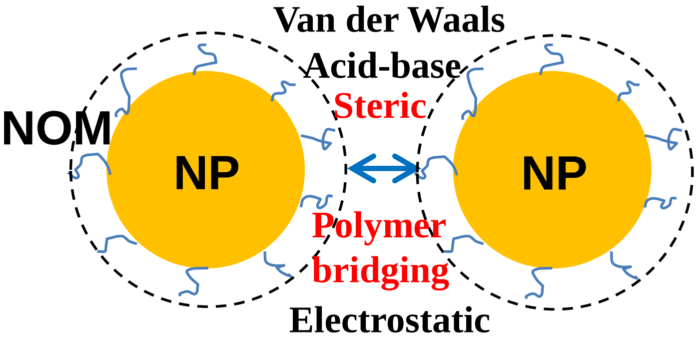




Kungang Li, Wen Zhang, Ying Huang, Yongsheng Chen. *J. Nanoparticle. Res*. 2011

2.2. Natural organic matter (NOM) effect on aggregation kinetics of NPs

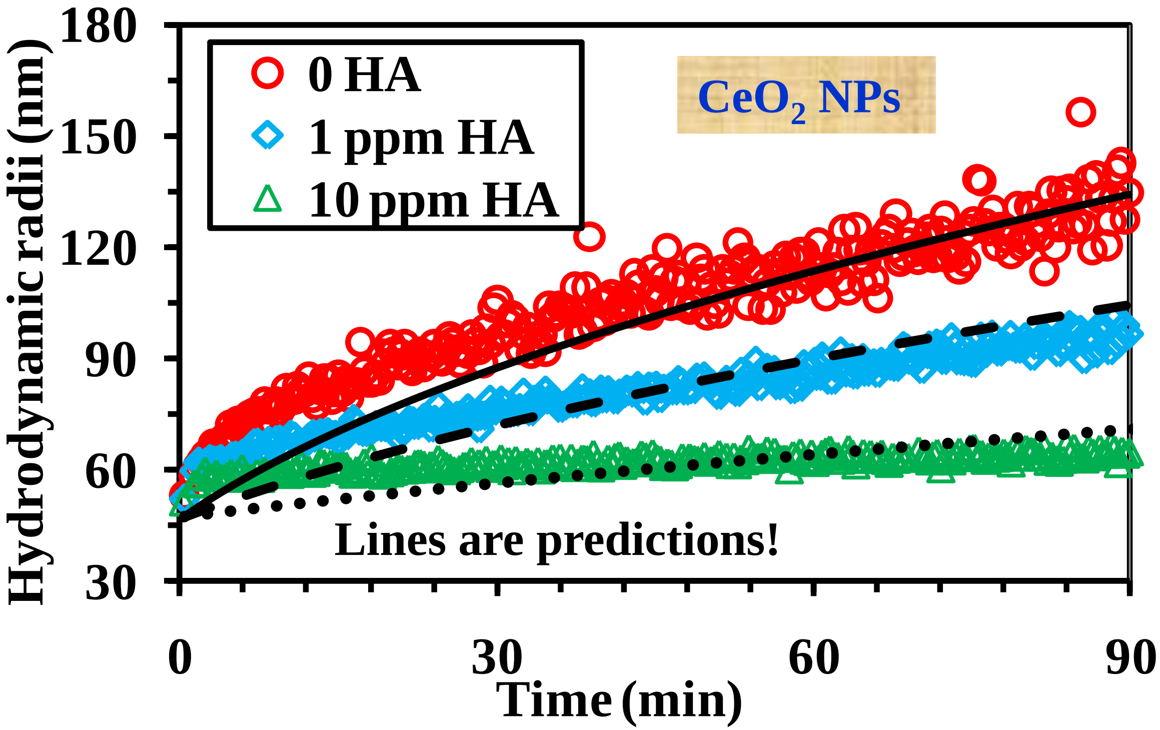




The adsorption of NOM molecules on NP surface will:

- **Change the vdW, AB, and EL interactions**
- **Introduce new interactions (e.g., steric and polymer bridging)**

$$r = a \cdot \left\{ 1 + \frac{4k_B T n_0}{3\enclose{circle}{\mu W}} t \right\}^{1/d_F}$$



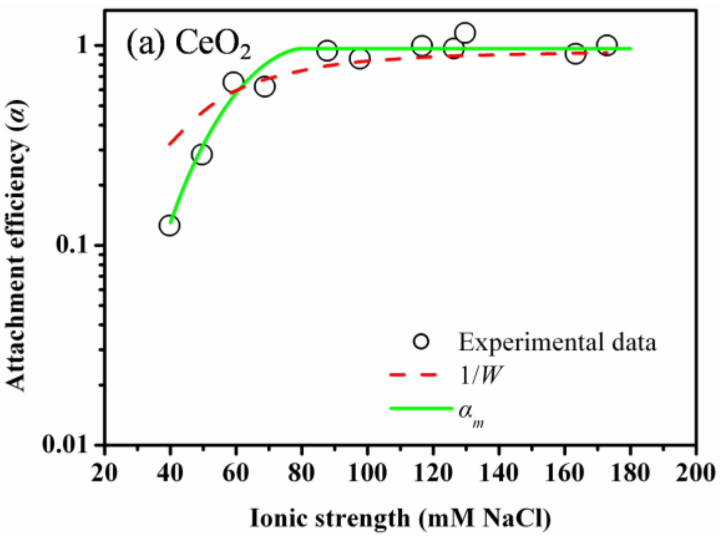
Kungang Li and Yongsheng Chen. *J. Hazard. Mater*. 2012

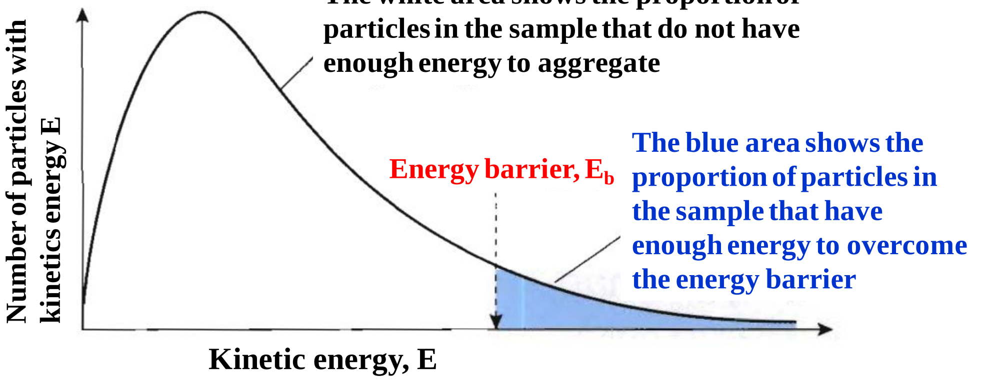
2.3. New attachment efficiency model on the basis of Maxwell-Boltzmann distribution

Conventional attachment efficiency equation

Limitation: neglecting Brownian motion or kinetic energy of NPs

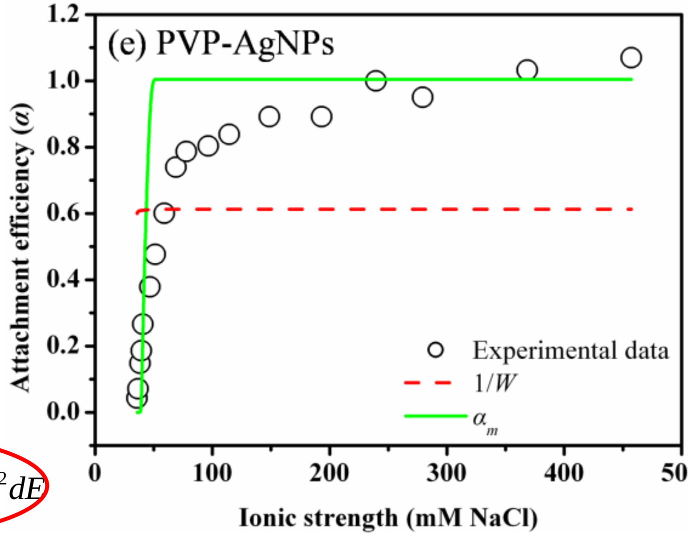
$$\alpha = \frac{\int_0^\infty \lambda(u) \frac{\exp\left(U_{iwi}^{vdw}(h)/k_BT\right)}{(2+u)^2} du}{\int_0^\infty \lambda(u) \frac{\exp\left(U_{iwi}^{DLVO}(h)/k_BT\right)}{(2+u)^2} du}$$





The white area shows the proportion of particles in the sample that do not have enough energy to aggregate

The blue area shows the proportion of particles in the sample that have enough energy to overcome the energy barrier



New attachment efficiency equation

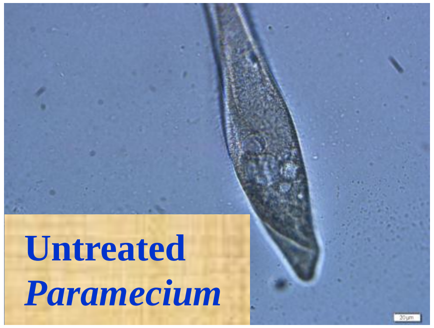
$$\frac{\Delta N_{E_b\to\infty}}{N_{0\to\infty}} = \frac{\int_{v_C}^\infty 4\pi\left(\frac{m}{2\pi k_BT}\right)^{3/2} e^{-\frac{mv^2}{2k_BT}} v^2 dv}{\int_0^\infty 4\pi\left(\frac{m}{2\pi k_BT}\right)^{3/2} e^{-\frac{mv^2}{2k_BT}} v^2 dv} = \frac{\int_{E_b}^\infty e^{-E}E^{1/2}dE}{\int_0^\infty e^{-E}E^{1/2}dE}$$

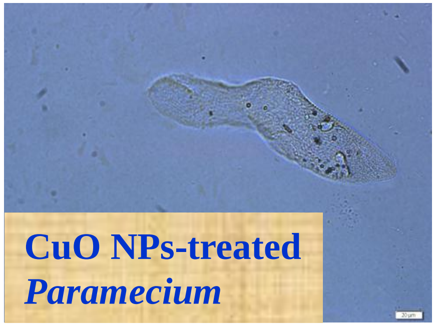
$$\boxed{\alpha_m = \delta\cdot\frac{\Delta N}{N} = \delta\cdot\int_{E_b}^\infty e^{-E}E^{1/2}dE}$$

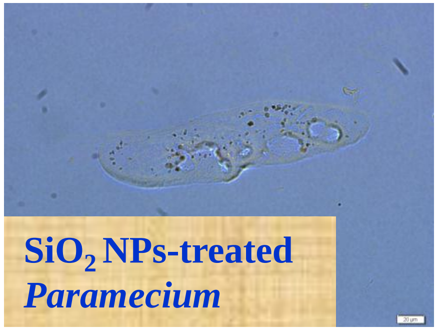
Wen Zhang, John Crittenden, Kungang Li, Yongsheng Chen. *Environ. Sci. Technol.* 2012

3.1. Acute toxicity results of NPs to *paramecium*

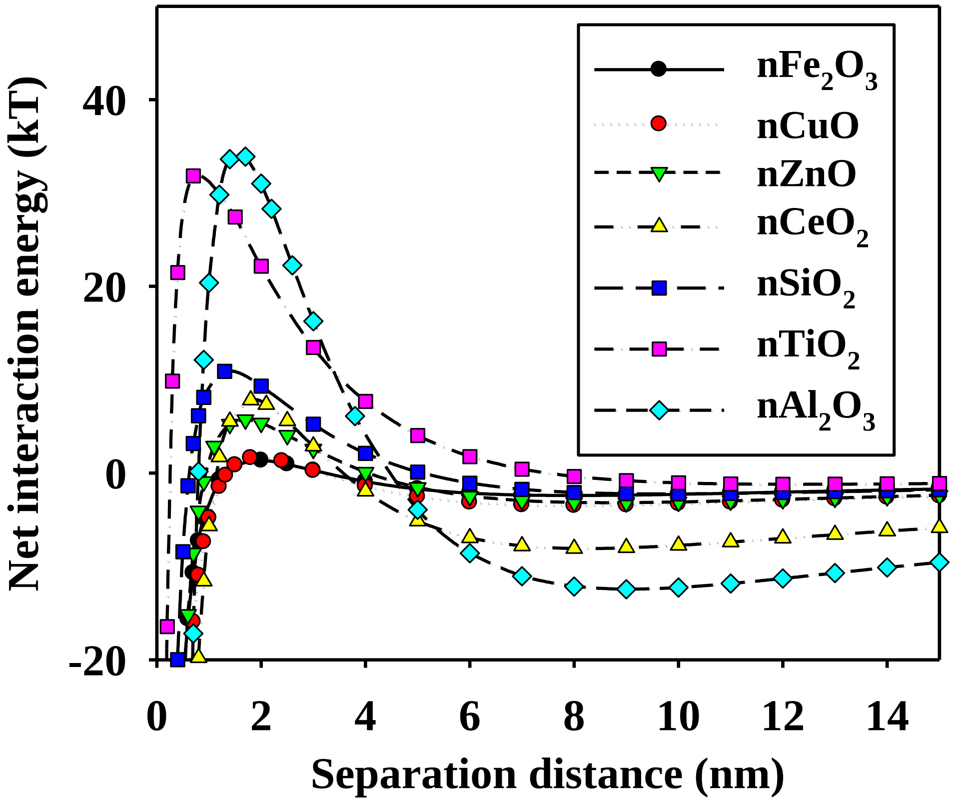
Metal oxide NPs	48-h LC_{50} (mg/L)	95% confidence intervals (mg/L)
nFe_2O_3	0.81	0.60–1.09
nCuO	0.98	0.84–1.25
$nSiO_2$	442.6	337.0–559.8
nZnO	573.8	448.6–707.9
$nCeO_2$	1832.5	1739.9–1925.1
$nTiO_2$	7215.2	3730.1–38142.7
nAl_2O_3	9269.2	4783.1–35409.6


Untreated *Paramecium*


CuO NPs-treated *Paramecium*

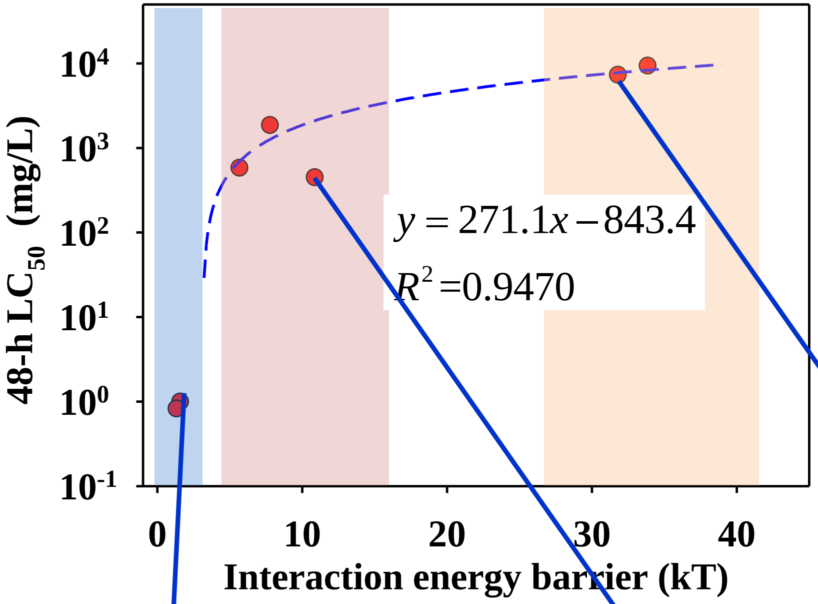

SiO_2 NPs-treated *Paramecium*

3.2. NP-cell interaction correlates with NP toxicity to *paramecium*

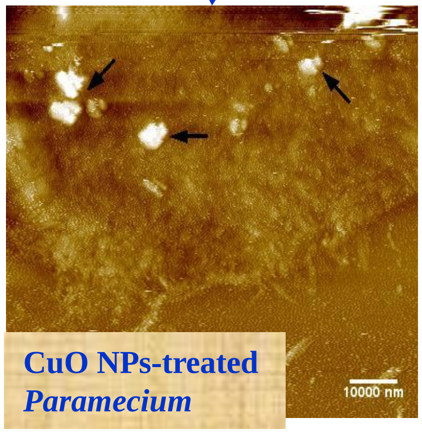


Interaction energy between NPs and *paramecium* cell membrane

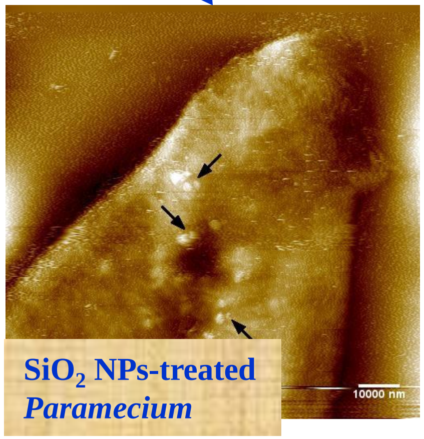
3.3. NP-cell interaction correlates with NP toxicity to *paramecium*



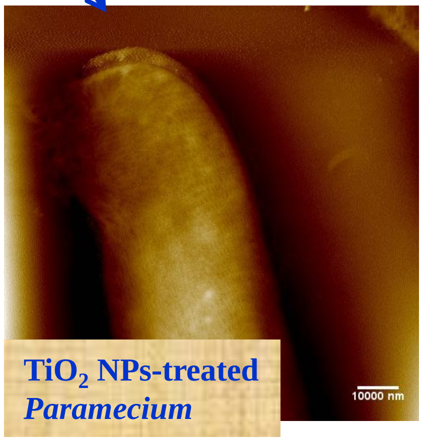
- **Interaction energy barrier is well correlated with the acute toxicity of NPs**
- **Low energy barrier implies stronger NP-cell association and thus more damage to the cell**
- **NP-cell surface interaction plays an important role in the cytotoxicity of NPs.**



CuO NPs-treated *Paramecium*

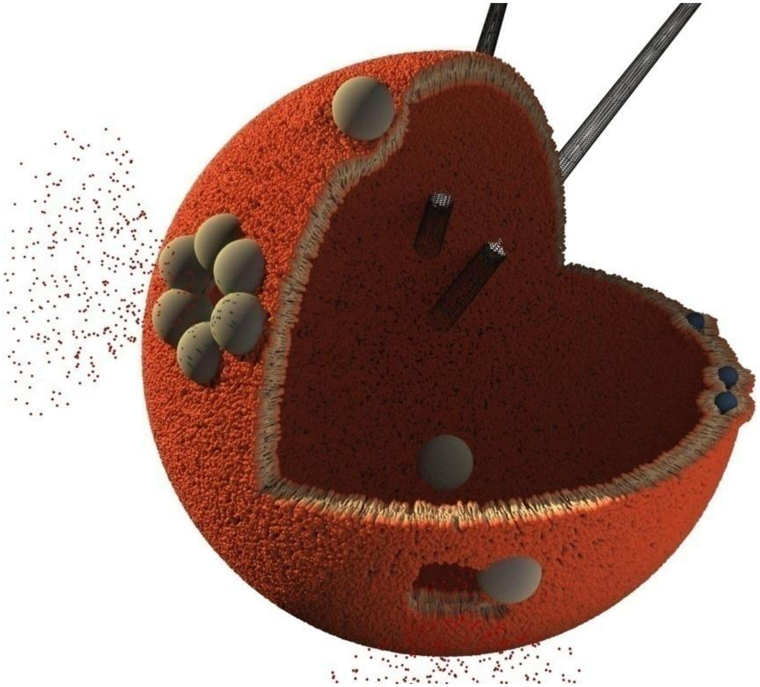


SiO_2 NPs-treated *Paramecium*



TiO_2 NPs-treated *Paramecium*

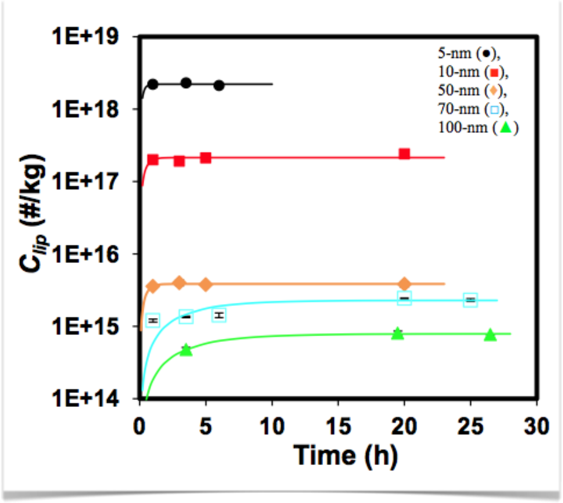
4.1. Using model cell membranes as predictor of bioaccumulation and toxicity.



Nanoparticles can have wide range of interactions with lipid bilayers that make up cell membranes (e.g. adsorption, partitioning, translocation, penetration, disruption)

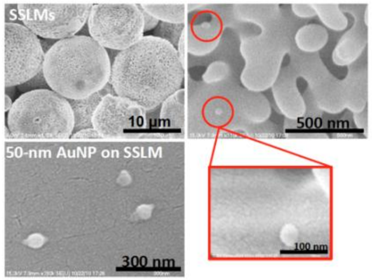
A. Negoda1, Y. Liu1, W-C Hou, C. Corredor, B.Y. Moghadam, C. Musolff, L. Li, W. W, P. Westerhoff, A.J. Mason, P. Duxbury, J.D. Posner, R.M. Worden.. Int. J. of Biomedical Nanoscience and Nanotechnology, submitted.
B.Y. Moghadam, W.C. Hou, C. Corredor, P Westerhoff, J.D. Posner. 2012. ACS Nano, Submitted.
C. Corredor, W-C Hou, S.A. Klein, B.Y. Moghadam, M. Goryll, P. Westerhoff, J.D. Posner. 2012. ACS Nano, Submitted.
W-C. Hou, B.Y. Moghadam C. Corredor, P. Westerhoff, J.D. Posner. 2012. Environmental Science and Technology, 46 (3), 1869–1876
W-C. Hou, B.Y. Moghadam, P.K. Westerhoff & J.D. Posner. 2011. Langmuir, 27(19), 11899–11905
K.D. Hristovski, P Westerhoff, J.D. POSNER. 2011. J. Envir. Sci. and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 46, 636-647.

4.2. Adsorption to lipid bilayers as predictor for toxicity and bioaccumulation

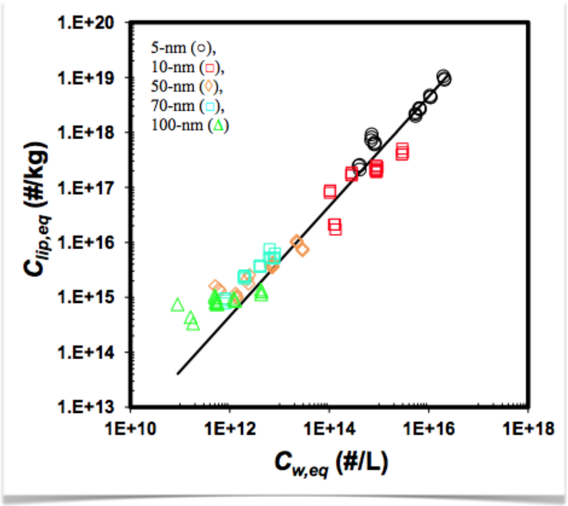


Adsorption rate of tannic acid coated Au NPs (9 mg/L) to DOPC bilayers.

- Small particles adsorb faster but to a lesser extent.
- Adsorption reaches some steady state (unsaturated <3% coverage) value in less than 10 hours.
- Why do particles stop adsorbing?

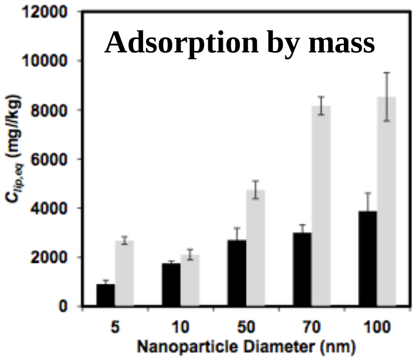


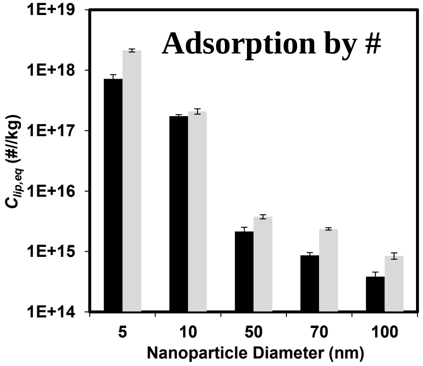
SEM images of Au NP on bilayer surface. Shows low coverage and isolated particles.



Steady state lipid bilayer-water distribution of tannic acid Au NP at pH 7.4, indicating the distribution based on number concentration.

- All data falls on single line K=450 L/kg.
- Data suggests that number density is correct dosimetry, but designing experiments with equivalent number density can be difficult when size is varied.

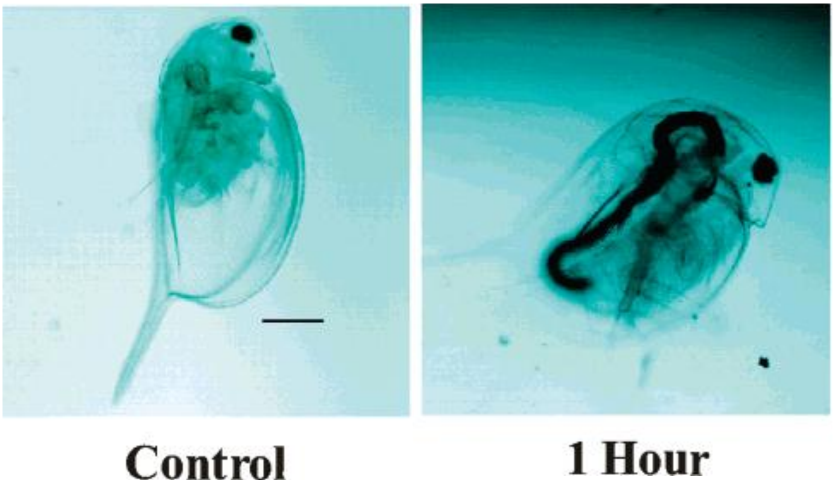




Interpretation of adsorption results depend on dosimetry choice. Single set of experiments suggest different outcomes based on presentation of dosimetry.

W-C. Hou, B.Y. Moghadam C. Corredor, P. Westerhoff, J.D. Posner. 2012. Environmental Science and Technology, 46 (3), 1869–1876
W-C. Hou, B.Y. Moghadam, P.K. Westerhoff & J.D. Posner. 2011. Langmuir, 27(19), 11899–11905
K.D. Hristovski, P Westerhoff, J.D. POSNER. 2011. J. Envir. Sci. and Health Part a-Toxic/Hazardous Substances & Envir. Eng., 46, 636-647.

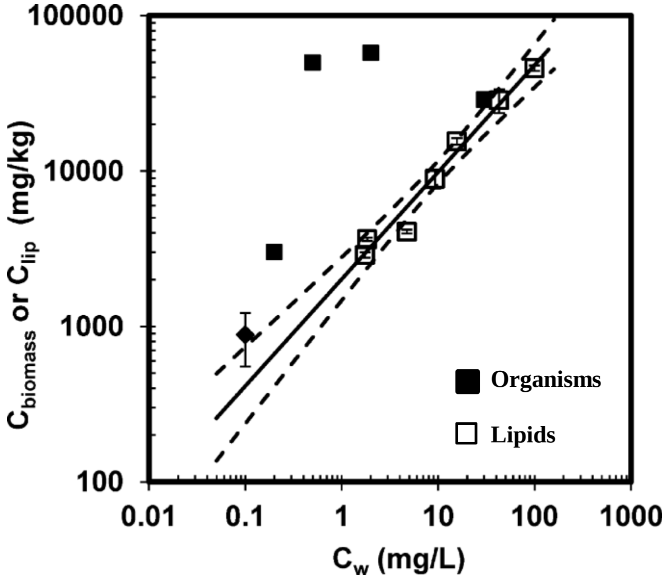
4.3. Adsorption to lipids correlates to bioaccumulation in aquatic organisms.



Control

1 Hour

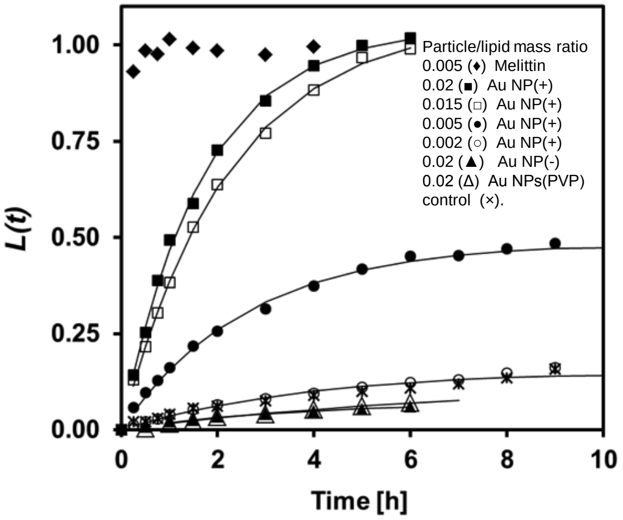
NPs accumulate in organisms. Single-walled carbon nanotubes accumulated in *Daphnia magna*. (Roberts et al., ES&T, 2007)



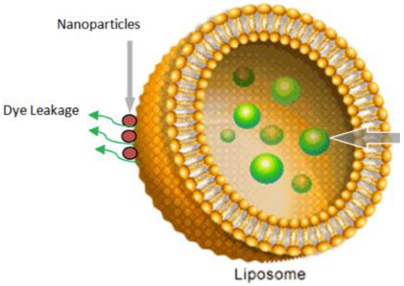
Preliminary data suggests that lipid bilayers can be used as a surrogate for predicting bioaccumulation in aquatic organisms. This is analagous to octanol-water partitioning approaches for molecular compounds.

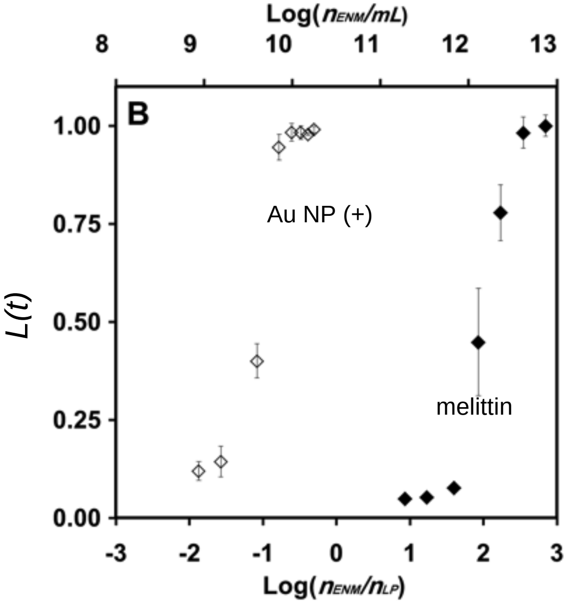
Negoda1, Y. Liu1, W-C Hou, C. Corredor, B.Y. Moghadam, C. Musolff, L. Li, W. W, P. Westerhoff, A.J. Mason, P. Duxbury, J.D. Posner, R.M. Worden.. Int. J. of Biomedical Nanoscience and Nanotechnology, submitted.
W-C. Hou, B.Y. Moghadam, P.K. Westerhoff & J.D. Posner. 2011. Langmuir, 27(19), 11899–11905

4.4. Nanoparticle disruption of model cell membranes

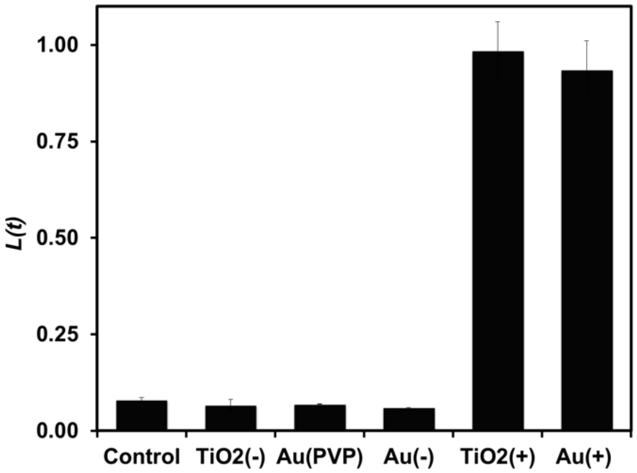


- NP induce leakage in liposomes exponentially over several hours.
- Can be fit with simple first order reaction model.
- Leakage increases with dosage.
- Positively charged NP induce faster leakage than negatively charged ones.

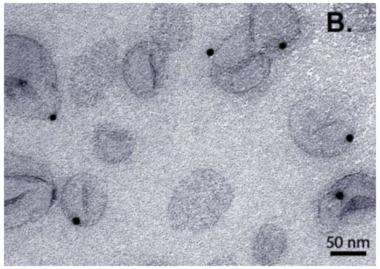




- Leakage as function of normalized concentration (number of particles per number of liposomes) shows sigmoidal behavior analogous to toxicity.
- Only one Au(+) NP is required per liposome to induce complete leakage.
- NP are more effective than melittin in disrupting bilayer (per particle)



- Leakage is strongly correlated with surface functionality
- Positive particles induce more leakage than negatively charged ones.



TEM confirming 1 particle per liposome

B.Y. Moghadam, W.C. Hou, C. Corredor, P Westerhoff, J.D. Posner. 2012. ACS Nano, Submitted.

C. Corredor, W-C Hou, S.A. Klein, B.Y. Moghadam, M. Goryll, P. Westerhoff, J.D. Posner. 2012. ACS Nano, Submitted.

Conclusions

1. ROS production by NPs

- **Al_2O_3 NPs were found to produce $^1O_2$ only, and CeO_2 NPs produced $O_2^{\bullet-}$ only.**
- **Energy band structures of metal oxide NPs could interpret the ROS production.**
- **NPs with a greater ROS production capability killed more *E.coli* cells.**

2. Aggregation of NPs

- **Non-DLVO interactions must be included for describing NP aggregation.**
- **Brownian motion is important for NP behavior in solution.**

3. Toxicity of NPs to *paramecium*

- **CuO and Fe_2O_3 are very toxic, while TiO_2 and Al_2O_3 NPs are essentially non-toxic.**
- **NP-cell interaction plays an important role in NPs' toxicity to *paramecium*.**

4. Model cell membranes as predictor of bioaccumulation and toxicity

- **Smaller Au NPs distribute onto lipid bilayers more rapidly than larger ones.**
- **Lipid bilayer-water distribution coefficient for Au NPs is 450 L/kg lipid.**
- **Lipid bilayer-water distribution is a potential method for assessing the bioaccumulation potentials of NPs.**
- **Positive NPs induce more leakage in liposomes than negatively charged ones.**
- **Only one Au(+) NP is required per liposome to induce complete leakage.**

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