A new concern of nanowaste: A case study of bacterial pathogenic evolution

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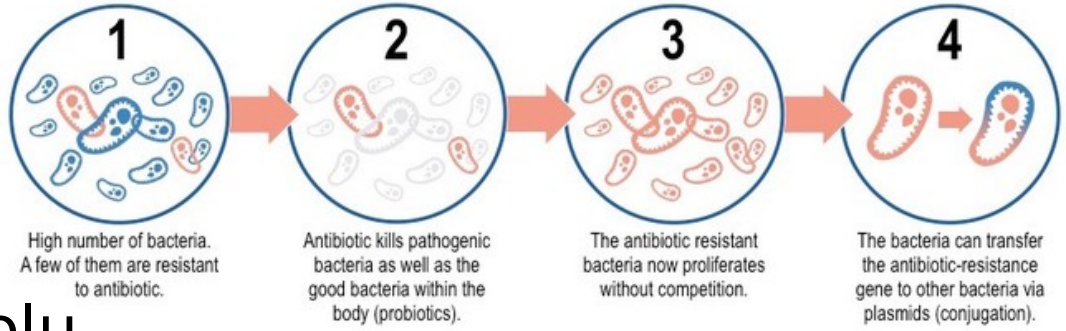
Background: Triclosan

Application

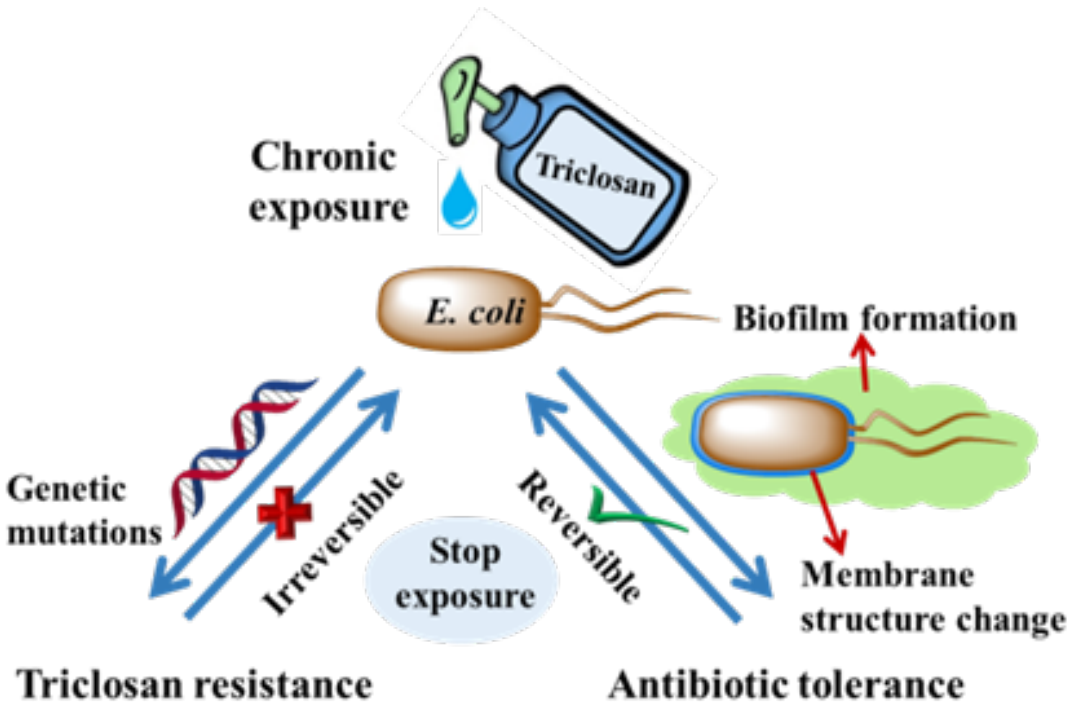
- Endocrine disruption
- Breast cancer
- Resistant bacteria
- Bioaccumulation
- Aquatic toxicity

Toxicity

Pathogenic selection/evolution

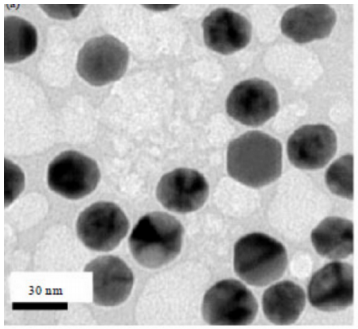


Background: Triclosan

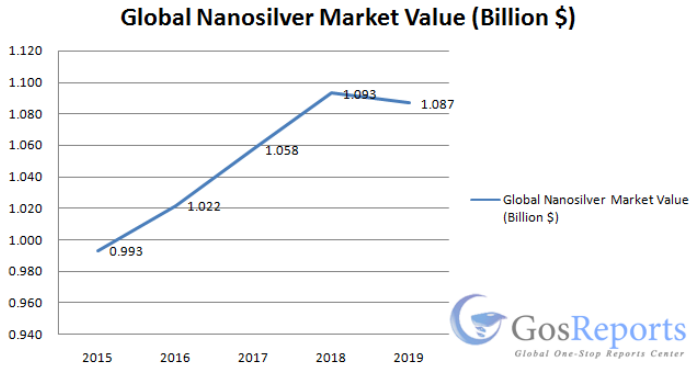


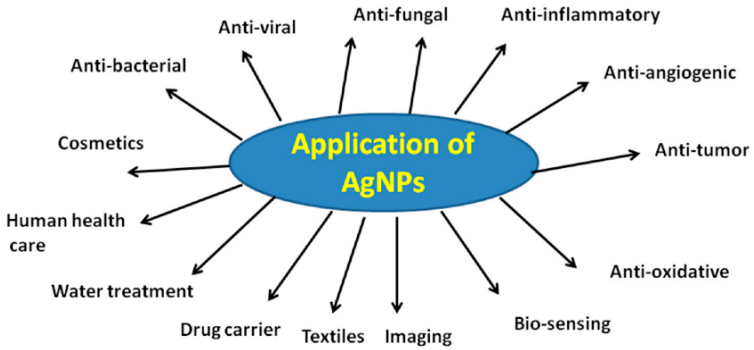
Zhang* et al., Environ Sci Technol, 2019

Background: nanosilver



- One of the promising substitutes is silver nanoparticle (AgNP).
- Potentially used in many daily life products for antimicrobial purpose.
- Increase of production, environmental release, ecological risk





Background: nanosilver

AgNP concentration in the environment

Table 1. Predicted Environmental Concentrations (PECs) of Highly Produced and Used Nanoparticles in Three Major Pathways in the Environment[a]

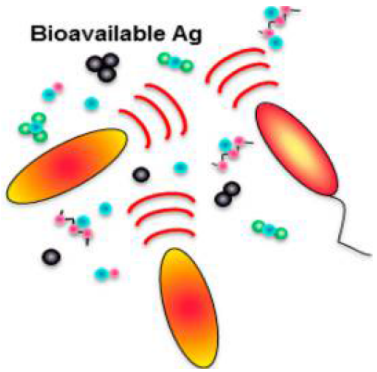
nanoparticle	PEC, pathway into environment	ref
Ag	0.088–10 000 ng/L, surface water	23, 25, 26, 28
	0.0164–17 μg/L, WWTP effluent	26, 28
	1.29–39 mg/kg, WWTP sludge	26, 28
TiO_2	21–10 000 ng/L, surface water	2, 23, 25, 26, 29, 30
	1–100 μg/L, WWTP effluent	26, 31, 32
	100–2000 mg/kg, WWTP sludge	26, 30, 31
ZnO	1–10 000 ng/L, surface water	26
	0.22–1.42 μg/L, WWTP effluent	26
	13.6–64.7 mg/kg, WWTP sludge	26
carbon-based	0.001–0.8 ng/L, surface water	23, 26
	3.69–32.66 ng/L, WWTP effluent	26
	0.0093–0.147 mg/kg, WWTP effluent	26

[a]WWTP: wastewater treatment plant.

(Anal. Chem., 2013)

Key Questions & Hypothesis

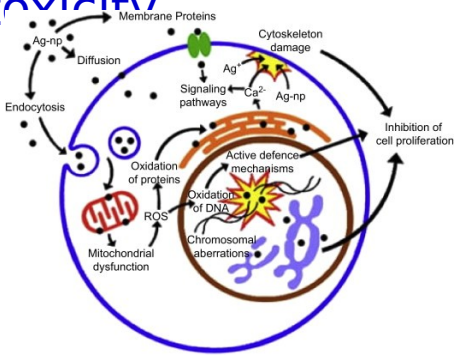
- Environmental exposure: low dose & long term
- is there selection on anti-silver microorganism?
- Will there be cross-resistance to antibiotics?
- Mechanism?



(ACS Nano, 2017)

Silver resistance determinants have increasingly been detected in a wide range of clinical and environmental microorganisms isolated from "ordinary" spots such as hospitals and industrial sites to "exclusive" locations including the water management systems of the International Space Station.

High dose, acute toxicity

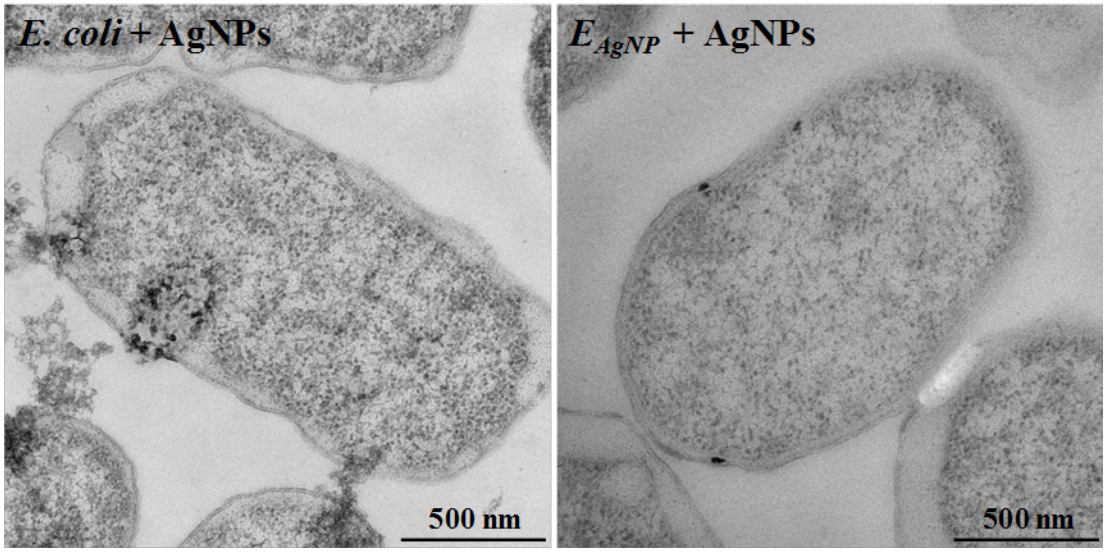


Experimental

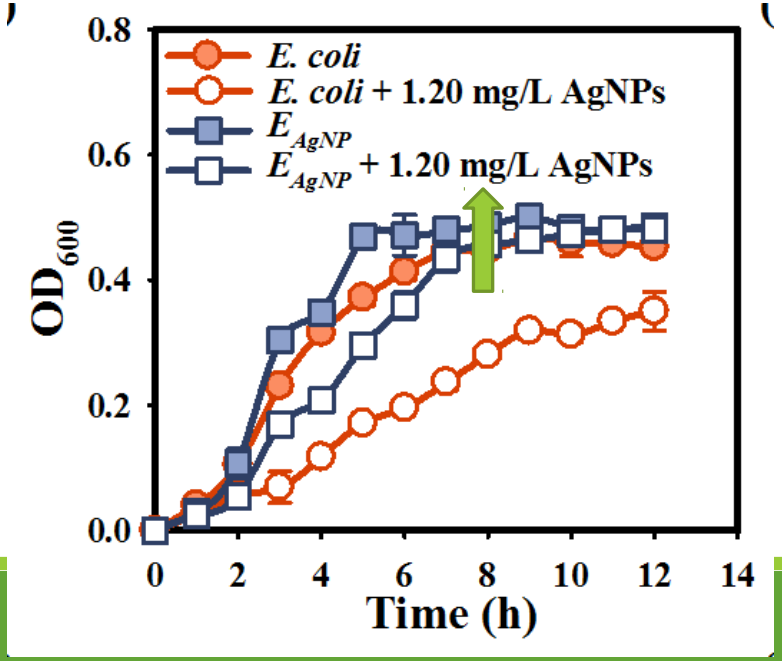
- AgNP characterization (Ag^+ or nanoparticle?)
- *E coli* was exposed to 0.02 μg/mL (1/100 MIC_{50}) of AgNP, for more than 200 subcultures (> 1yr).
- Monitor the changes in phenotypes (i.e., morphology, growth rate, change of minimal inhibition concentration MIC)
- illustrate the adaptive mechanism (based on transcriptomic and genomic analysis)
- Potential cross-resistance to antibiotics

Results and discussion

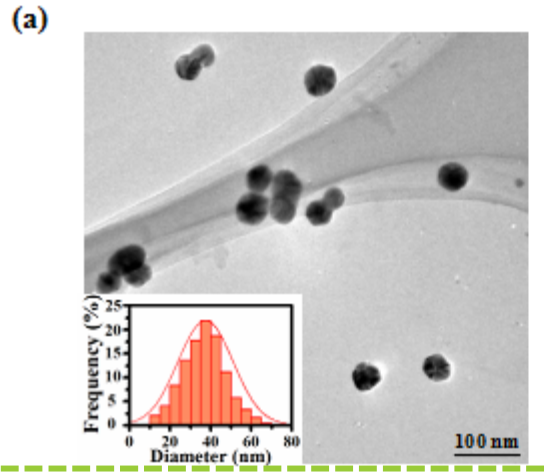
- No obvious morphological change

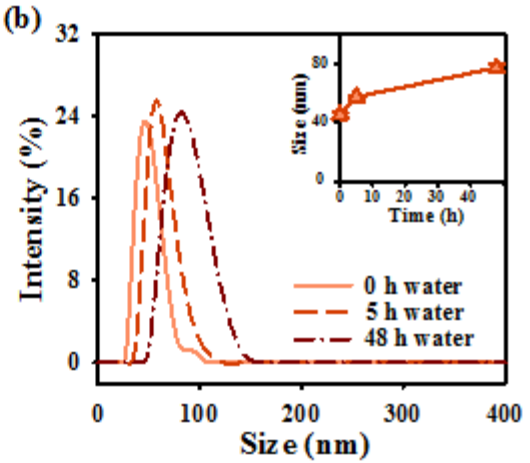


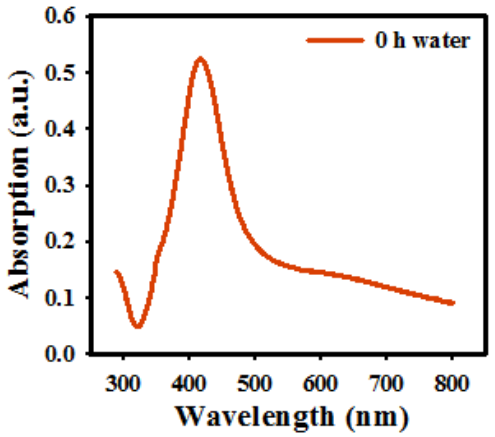
- After acclimation, no growth inhibition in response to ½ MIC



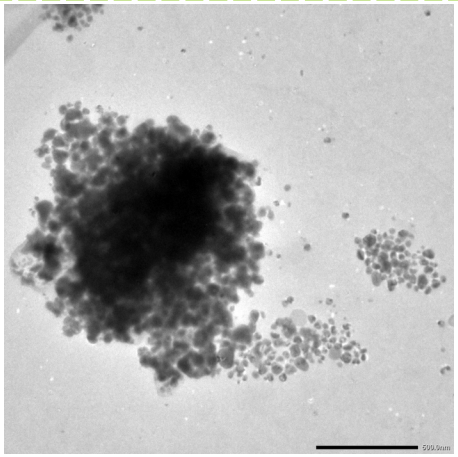
1st question: What are the working species? Silver ion or nanoparticles?

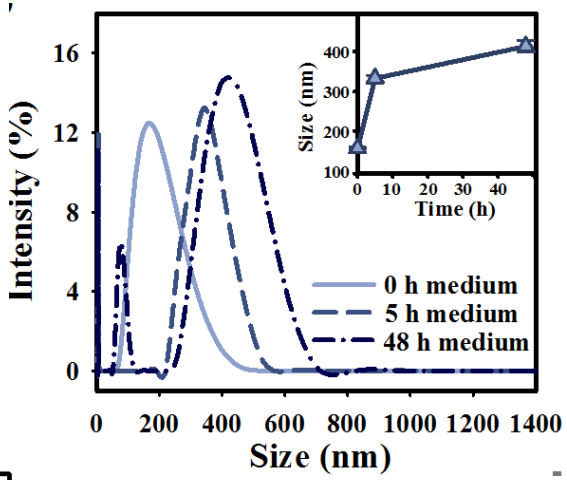


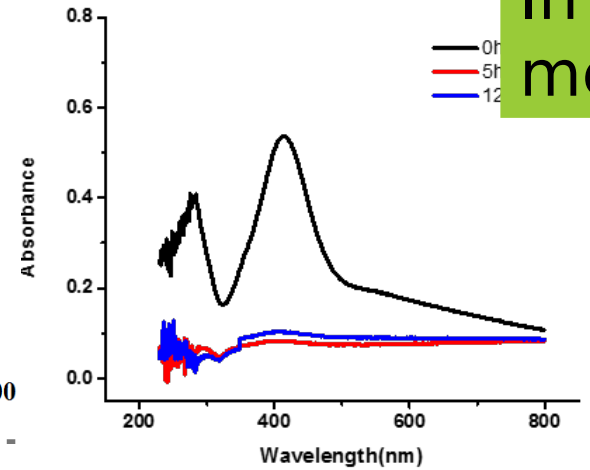




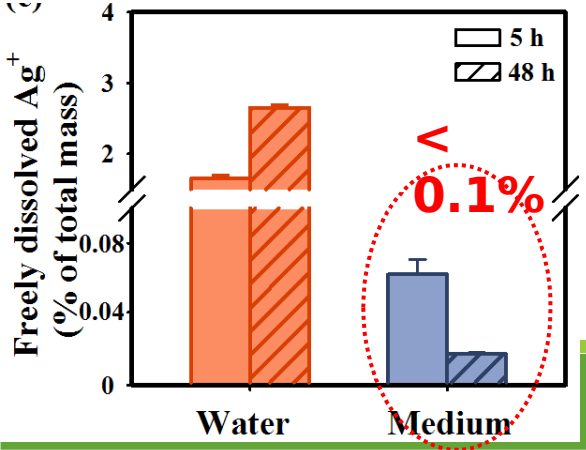
In water





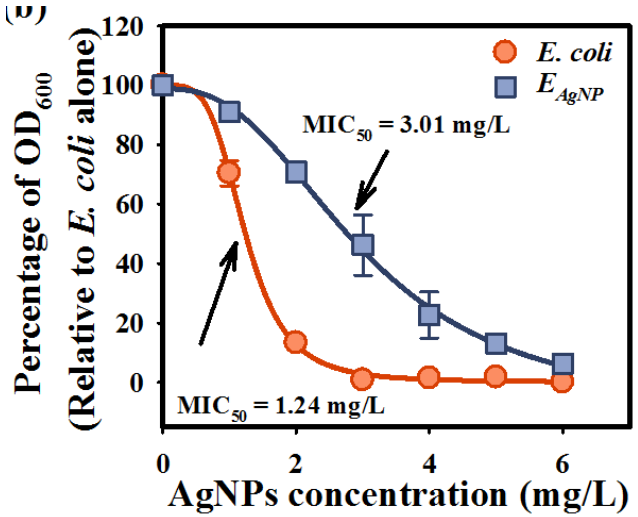


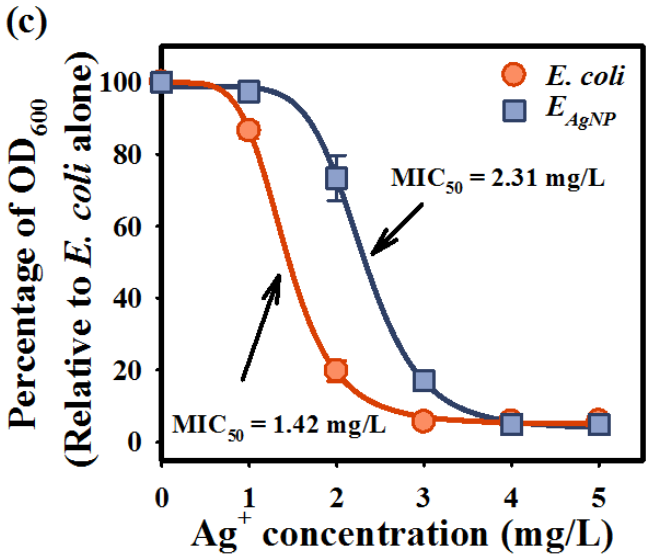
In medium



- few freely dissolved ions
- Protein Coated
- Aggregate

2nd question: anti-silver ion or nanoparticle? Mechanism?

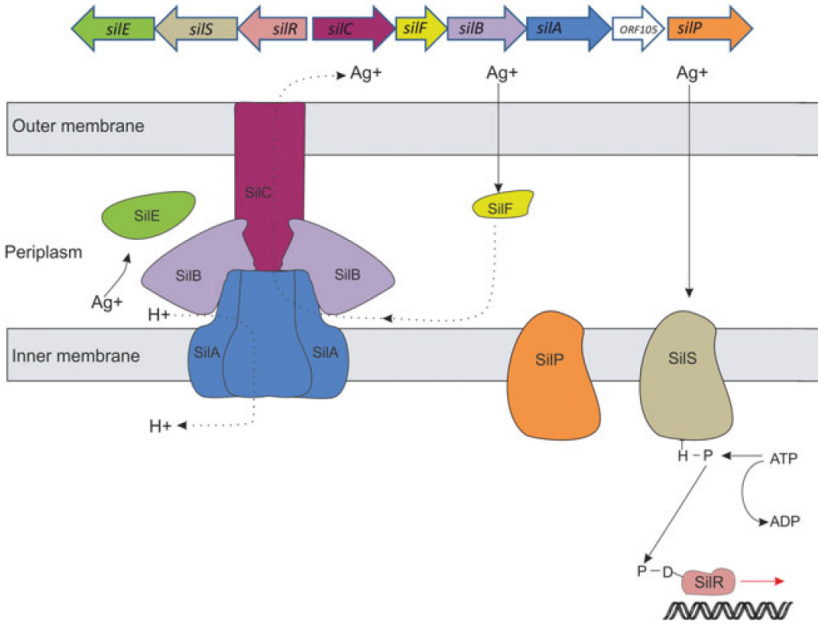


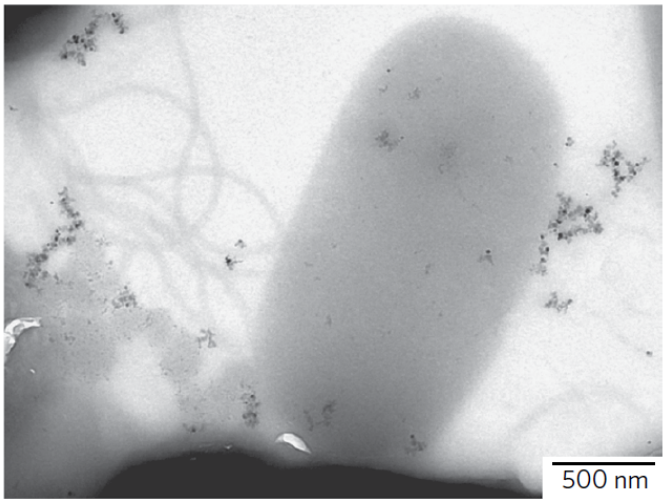


- Tolerance to both Ag^+ and Ag nanoparticle

Anti- Silver ion

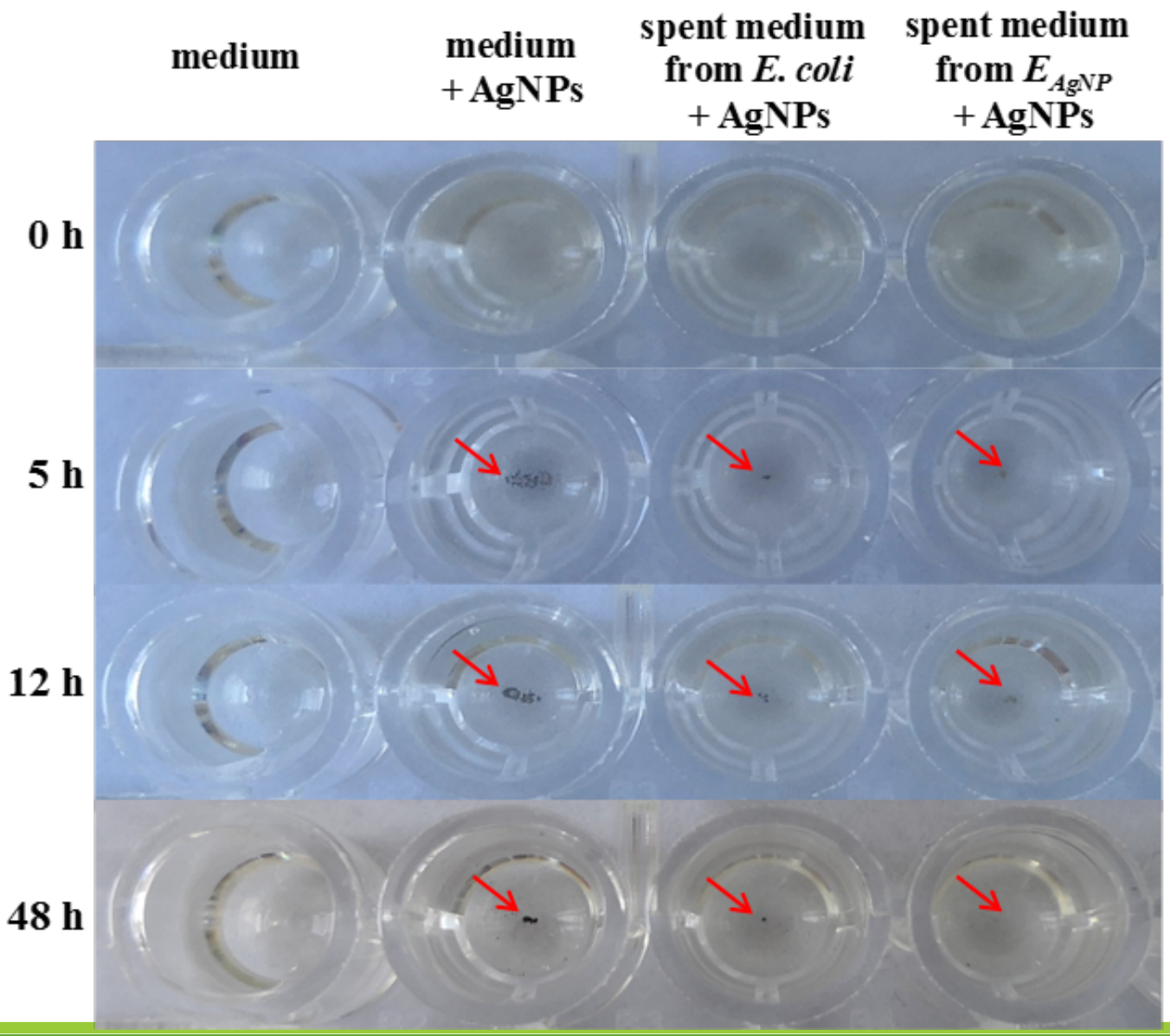
Anti-nanoparticle





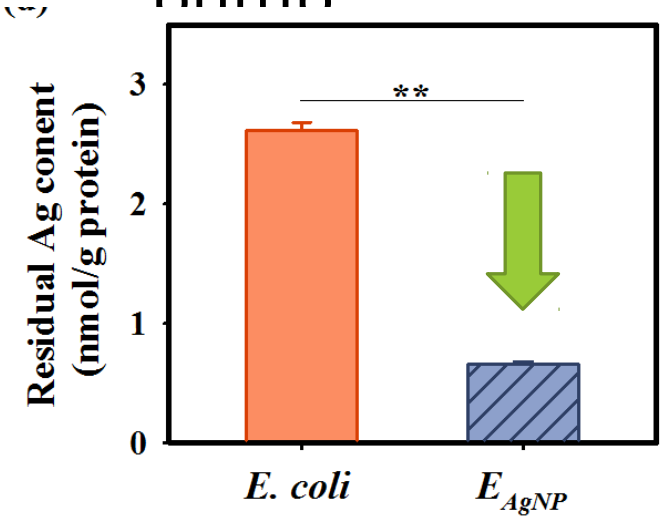
Ion efflux pump

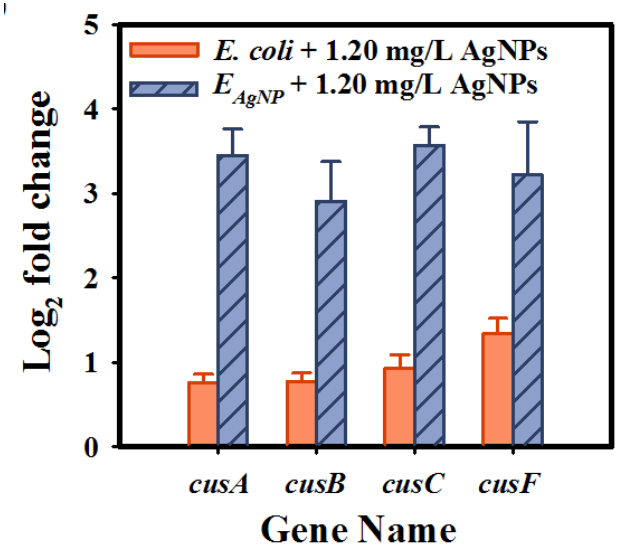
Extracellular precipitation

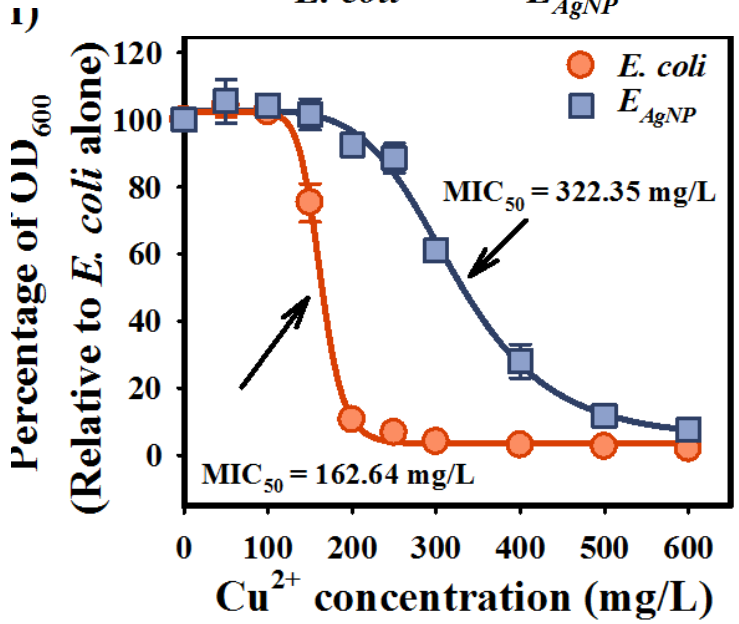


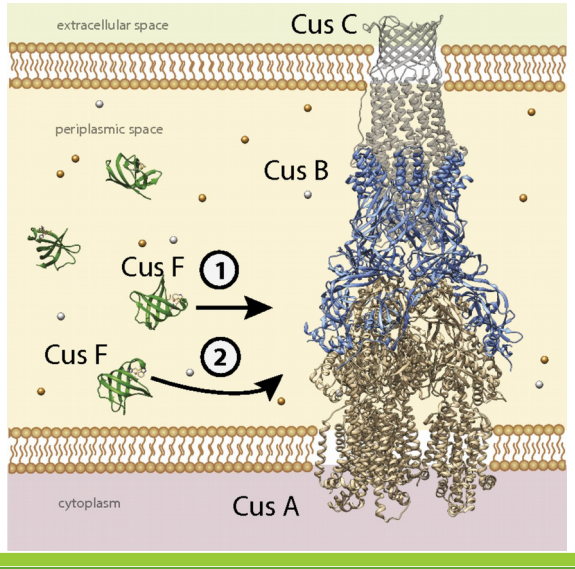
- No anti-nanoparticle effect

- Anti-silver ion via Cu-efflux pump



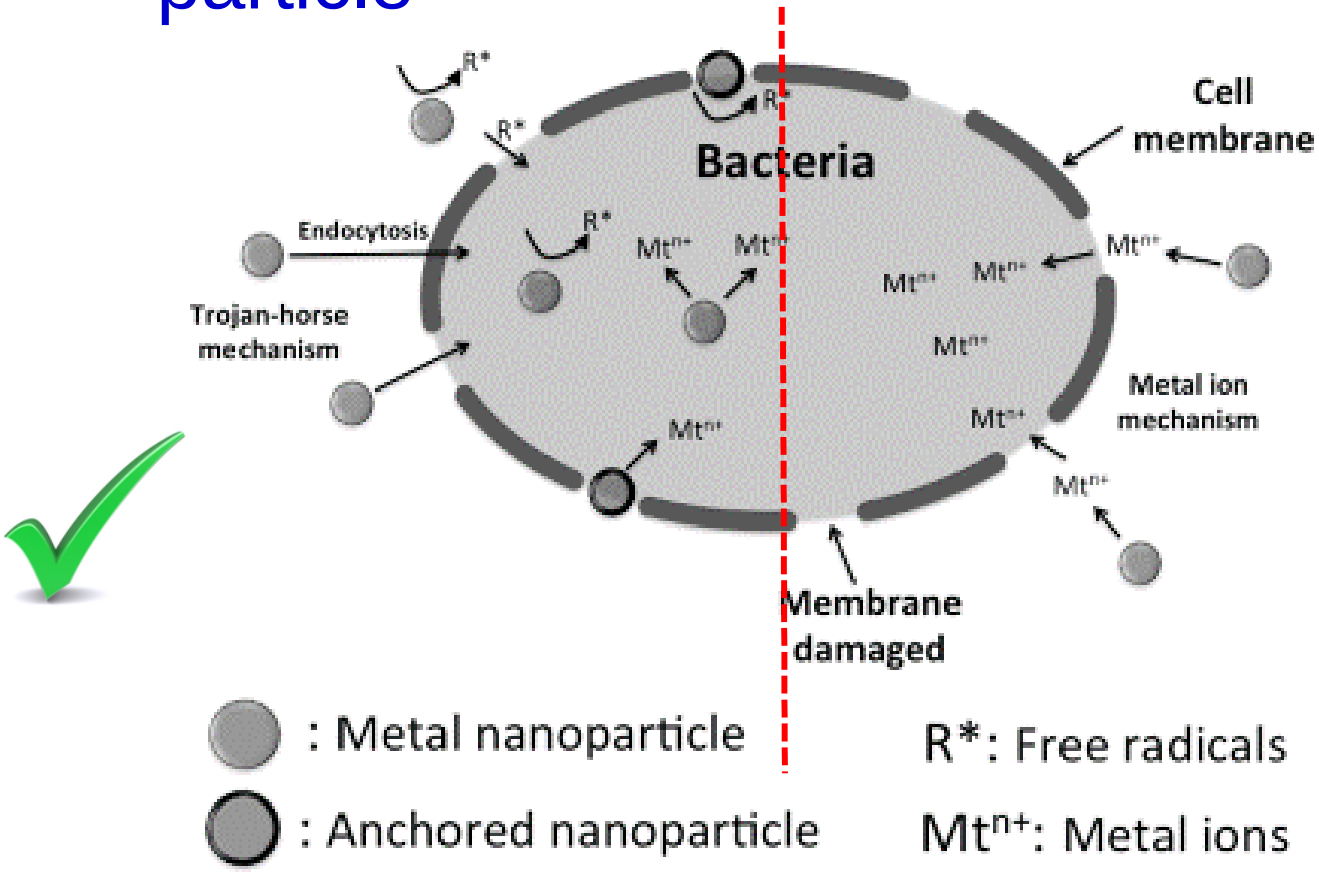






particle

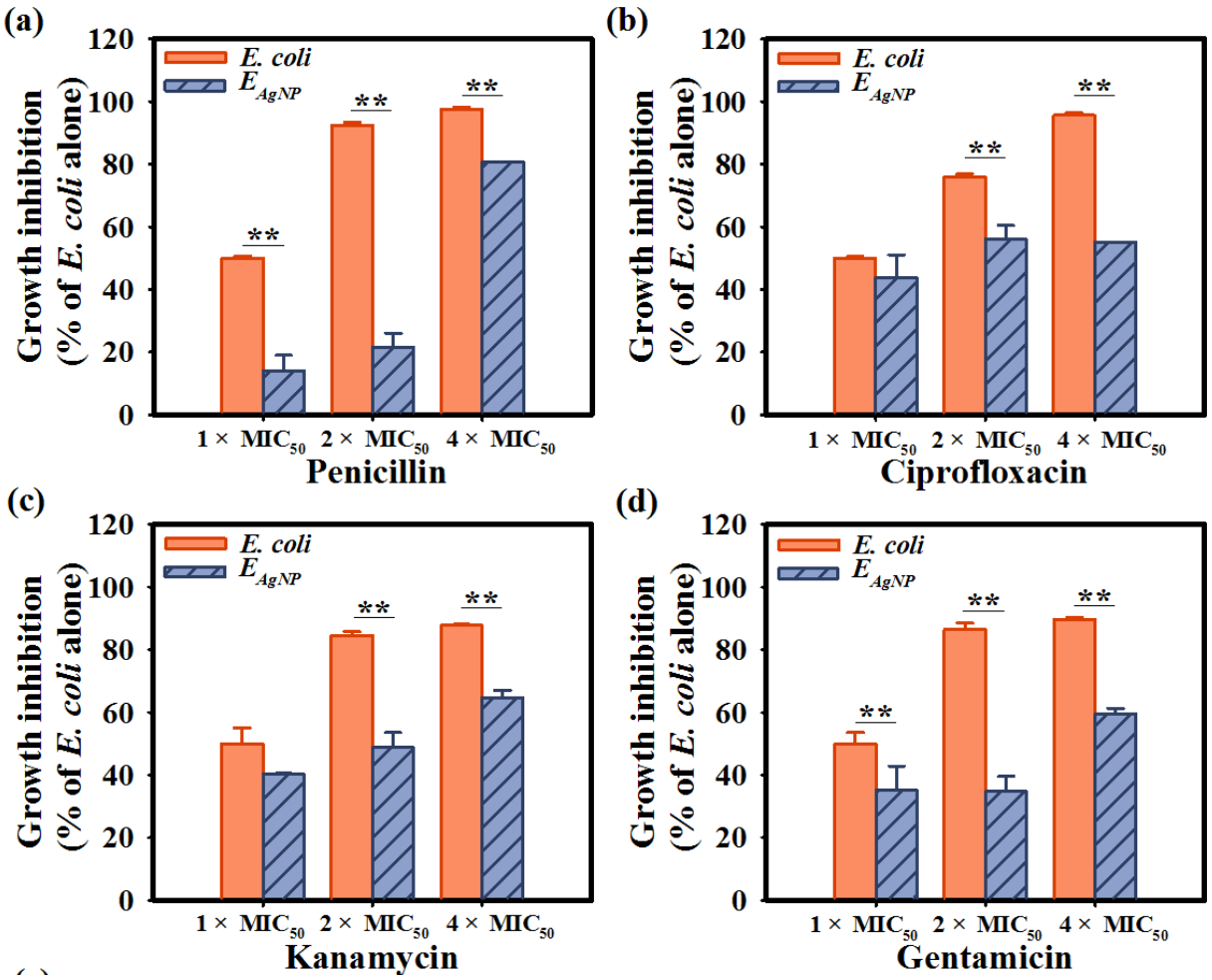
Ion



- Enter cell via Trojan-horse mechanism
- Release ion inside cells
- Develop adaptive response in order to eliminate intracellular silver ion

3rd question: Is there co-selection on antibiotic tolerance? Mechanism?

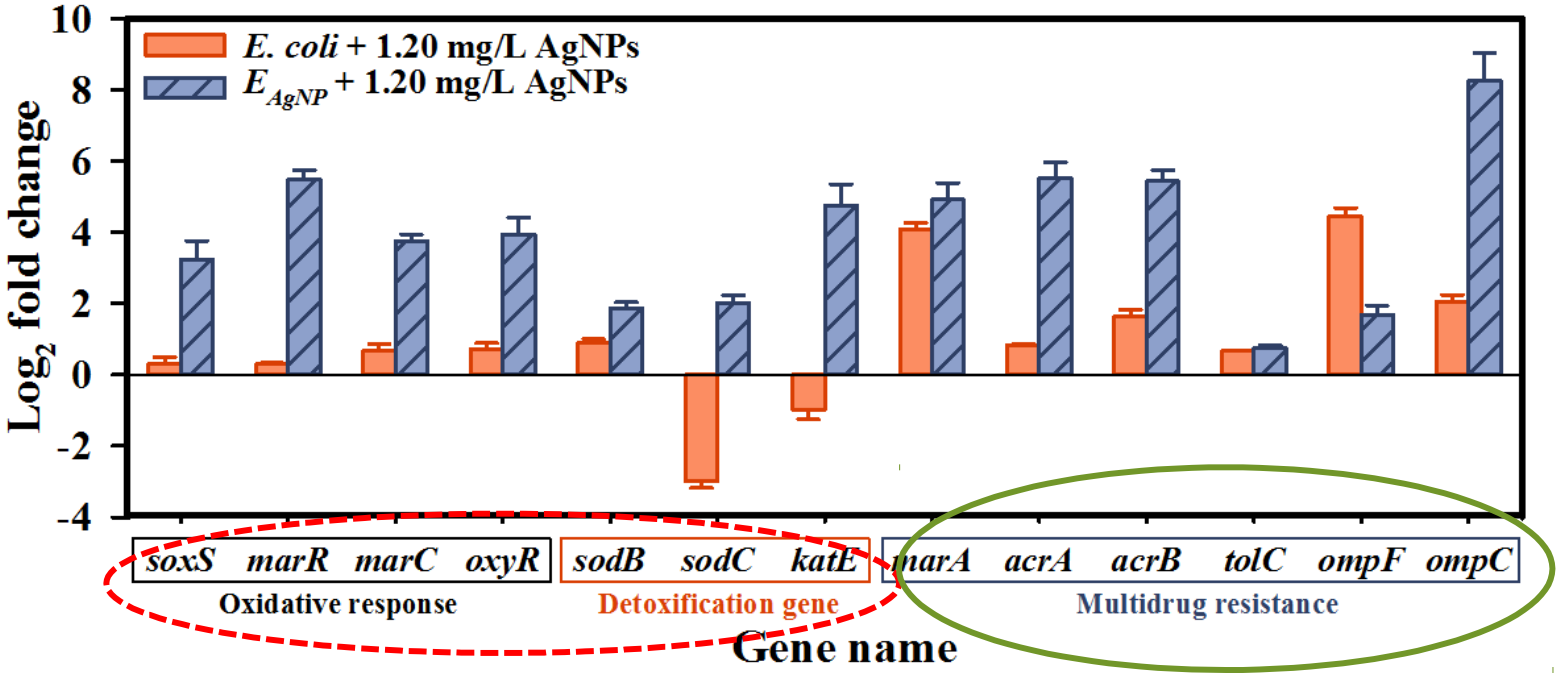
Cell wall



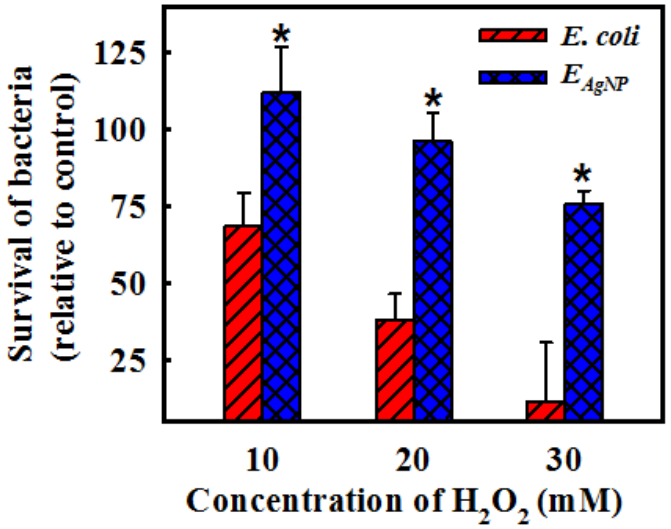
DNA/RNA synthesis

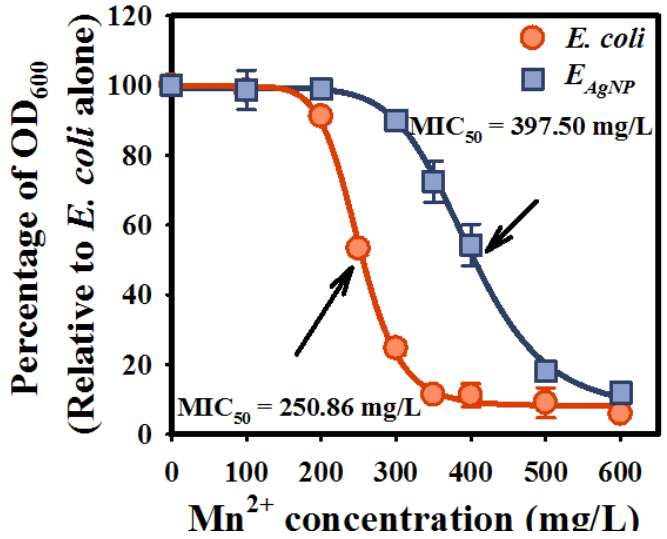
Protein synthesis

Protein synthesis



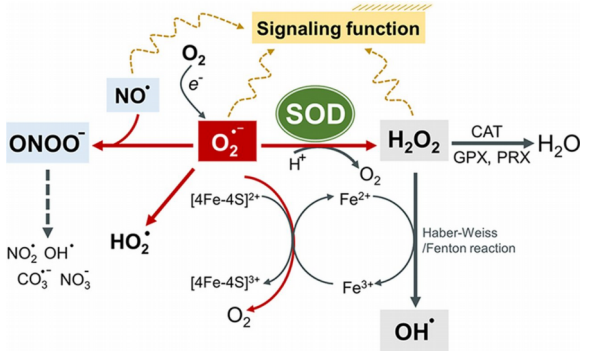
- Over-expression of multidrug resistance genes (efflux pump, porins).

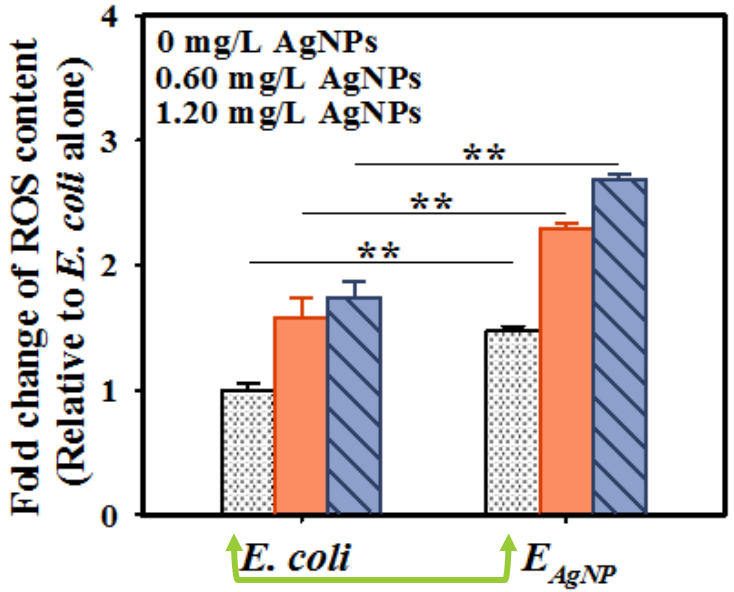




Multi-species tolerance:

- ✓ Ag^{+}, Ag nanoparticles, Cu^{2+}
- ✓ H_2O_2, Mn^{2+}
- ✓ multi-drugs





- High ROS content in adapted cells
- Without NP exposure, ROS content increase ~50% in adapted cells relative to wild type.

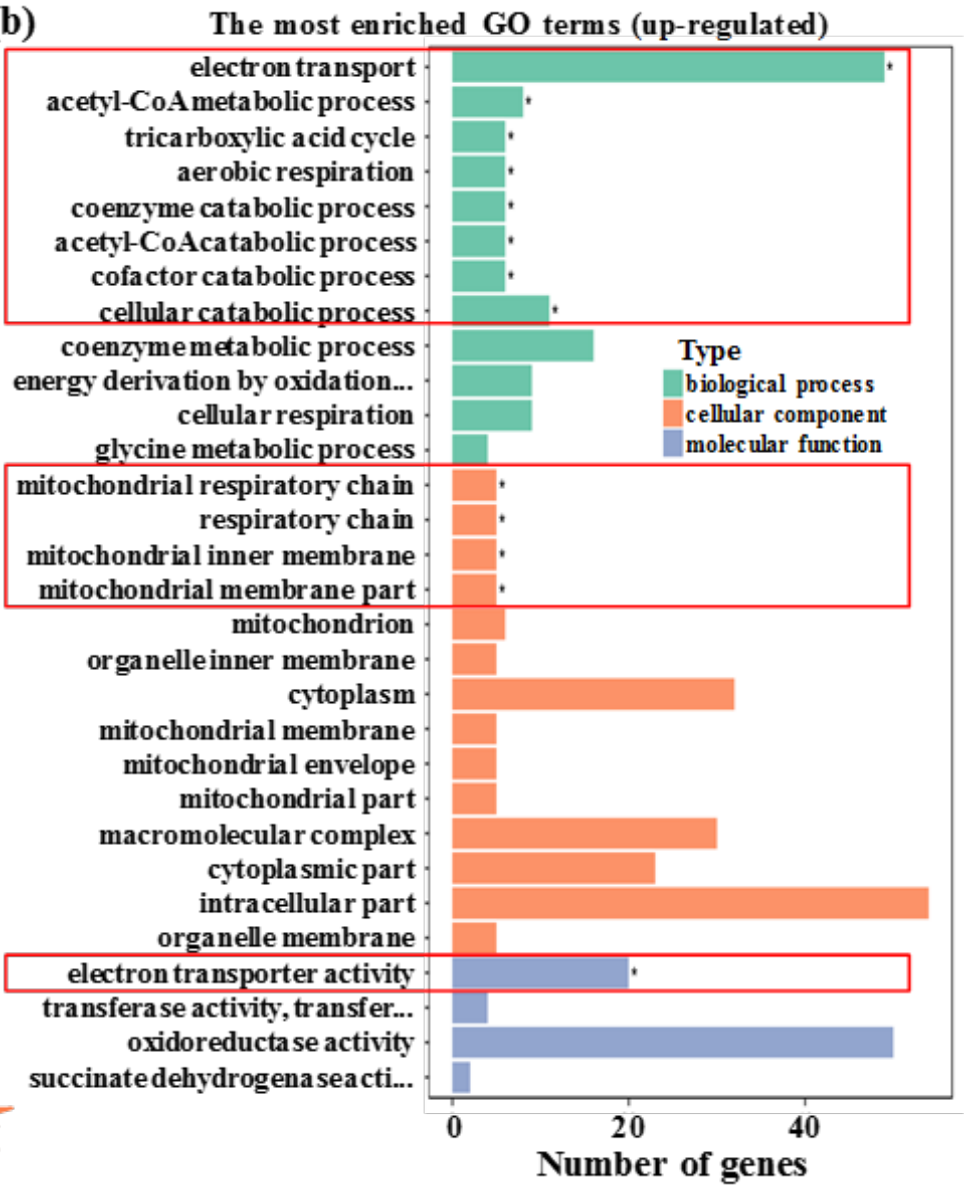
Oxidative stress response

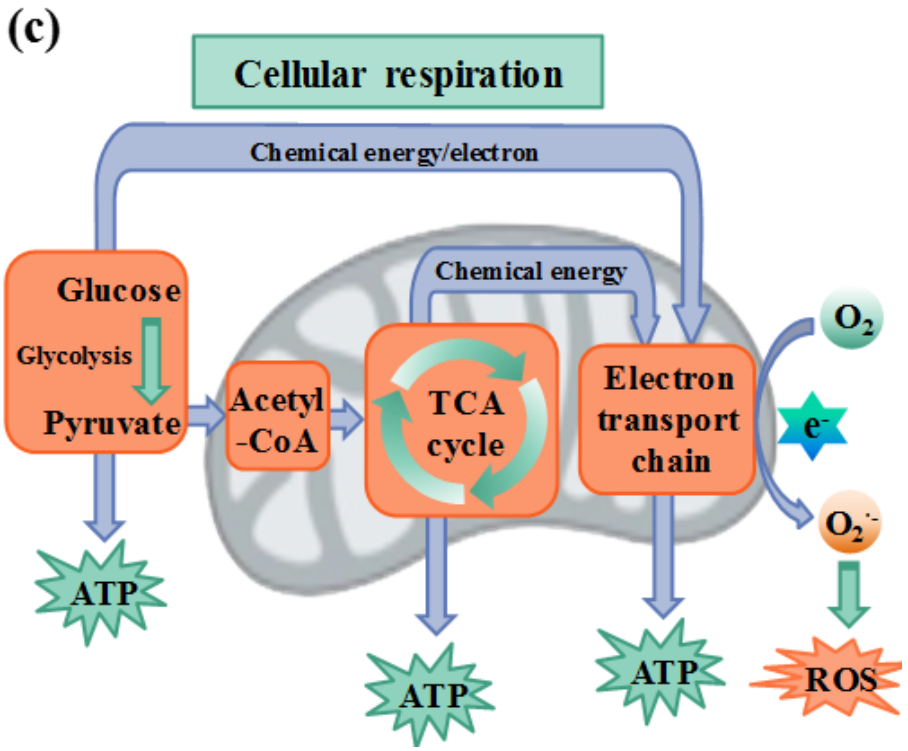
Activation of regulators

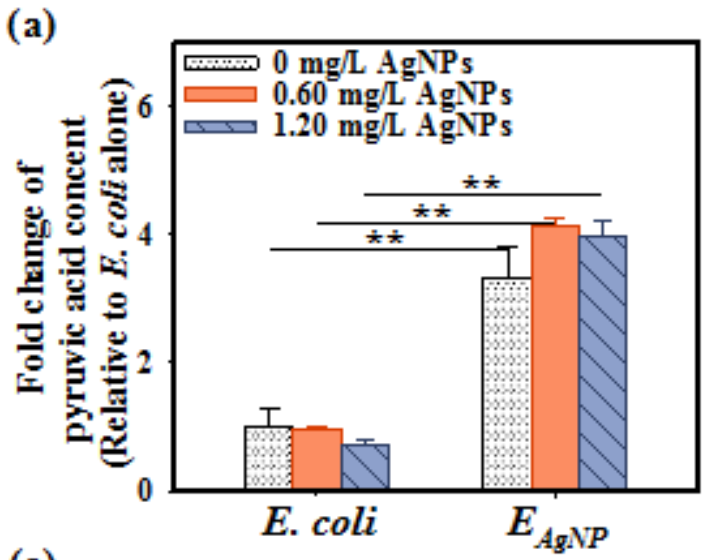
Up-regulation of scavenging enzymes

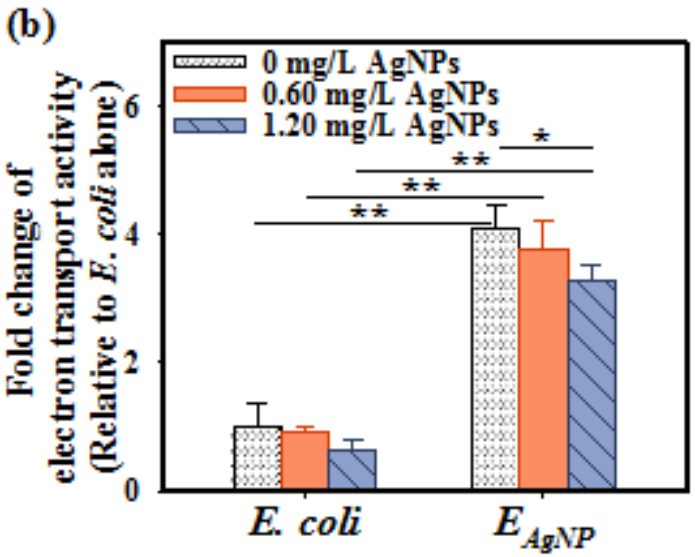
Repair of cellular damage

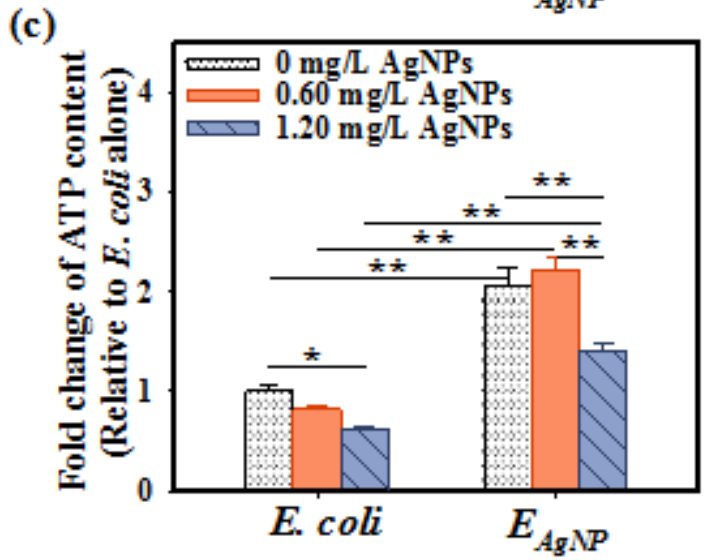
- No ROS production under acute exposure to 0.02 mg/L AgNPs
- High content of ROS after chronic exposure


b)
The most enriched GO terms (up-regulated)
electron transport
acetyl-CoA metabolic process
tricarboxylic acid cycle
aerobic respiration
coenzyme catabolic process
acetyl-CoA catabolic process
cofactor catabolic process
cellular catabolic process
coenzyme metabolic process
energy derivation by oxidation...
cellular respiration
glycine metabolic process
mitochondrial respiratory chain
respiratory chain
mitochondrial inner membrane
mitochondrial membrane part
mitochondrion
organelle inner membrane
cytoplasm
mitochondrial membrane
mitochondrial envelope
mitochondrial part
macromolecular complex
cytoplasmic part
intracellular part
organelle membrane
electron transporter activity
transferase activity, transfer...
oxidoreductase activity
succinate dehydrogenase acti...
Type
biological process
cellular component
molecular function
0
20
40
Number of genes


(c)
Cellular respiration
Chemical energy/electron
Chemical energy
Glucose
Glycolysis
Pyruvate
Acetyl -CoA
TCA cycle
Electron transport chain
ATP
ATP
ATP
O_2
e^-
$O_2^{\cdot-}$
ROS







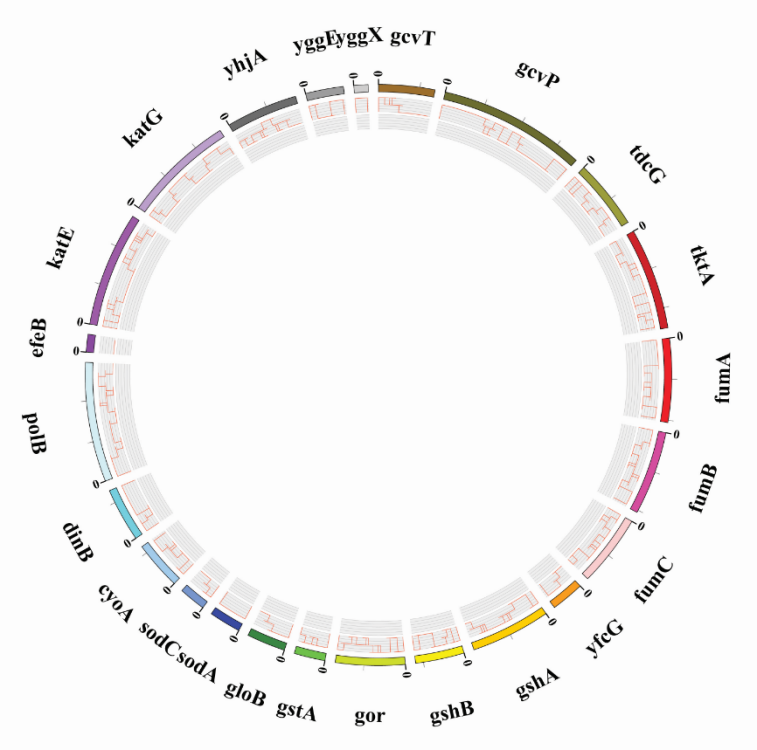
- Changes of physiological status

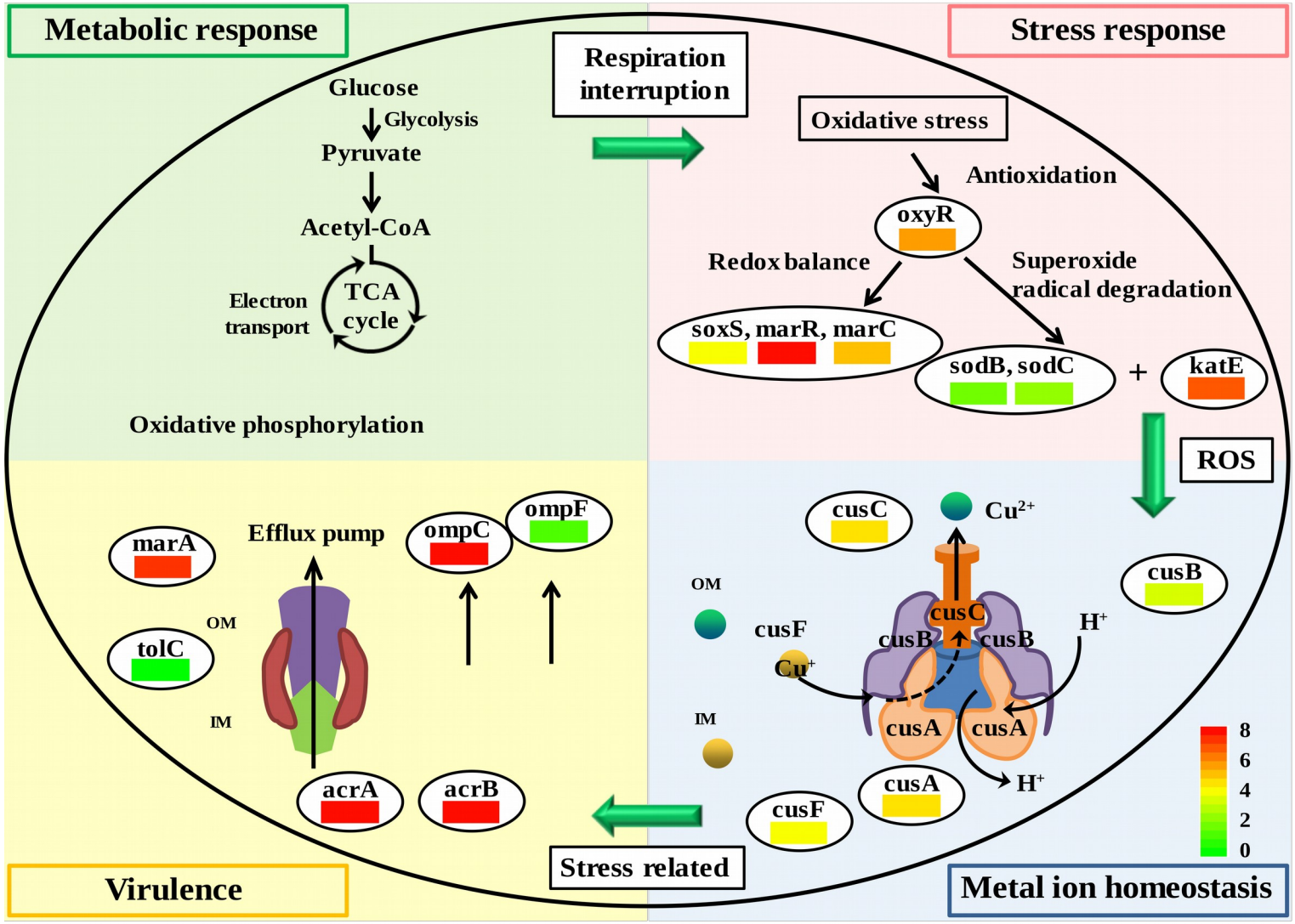
Oxidative stress induced DNA instability

Enriched functions potential related to virulence development:

- High mutation rate
- Mutations at :

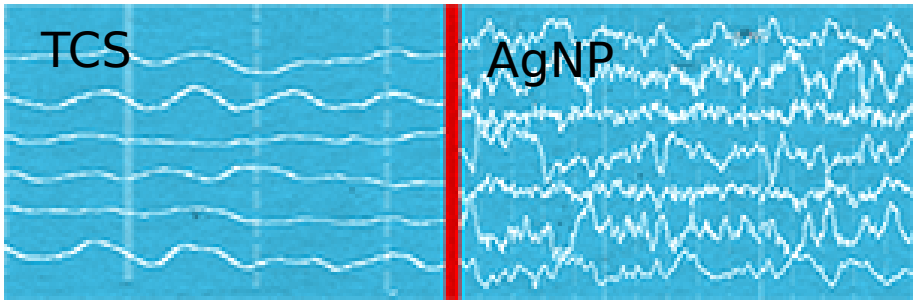
copper efflux pump
general stress
DNA repair (SOS)
antioxide



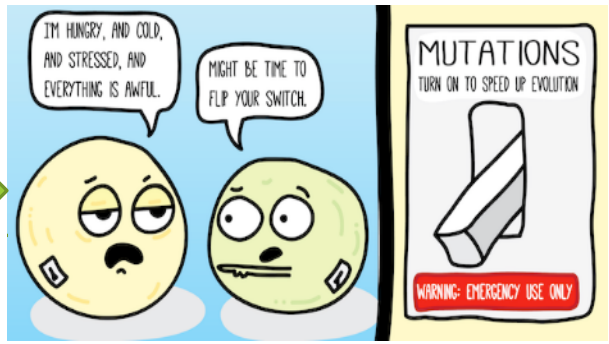

Metabolic response
Stress response
Respiration interruption
Glucose
Glycolysis
Pyruvate
Acetyl-CoA
Electron transport
TCA cycle
Oxidative phosphorylation
Oxidative stress
Antioxidation
oxyR
Redox balance
Superoxide radical degradation
soxS, marR, marC
sodB, sodC
+
katE
ROS
marA
Efflux pump
ompC
ompF
OM
tolC
IM
acrA
acrB
cusC
Cu2+
cusB
OM
cusF
cusC
cusB
cusB
H+
Cu+
IM
cusA
cusA
cusA
H+
cusF
8
6
4
2
0
Stress related
Virulence
Metal ion homeostasis

Conclusions

- Bacterial evolution was observed upon chronic exposure to low dose of AgNP.
- Co-development of multi-species tolerance (metal ions, nanoparticle, antibiotics, and oxidant) due to intracellular ROS stress.
- Stress-related DNA instability, and high mutation rate.
- Are we making a future superbug?



tranquil intense



Acknowledgement

- $$$ from NSFC (21777077) ；
- Graduate students: Mingzhu Li, Jing Li, Jing Sun

- ROS stress in evolved cells
- Multi species tolerance
- DNA instability