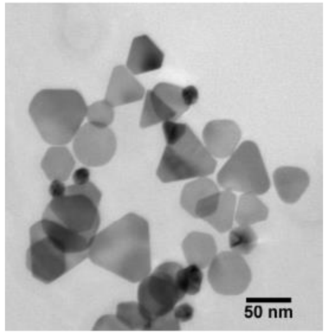
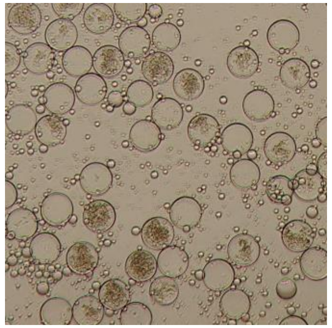
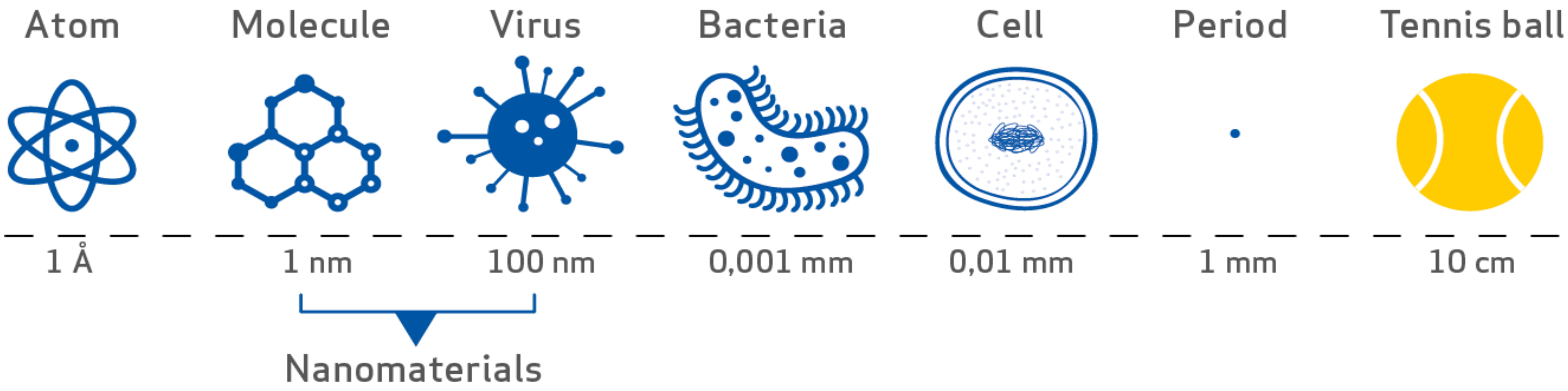
Investigation of antimicrobial silver nanomaterials from new angles: ***resistance, shapes, and single-cell level***

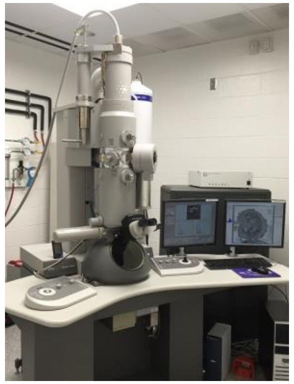
Chengfang Pang Kamp
23-09-2014



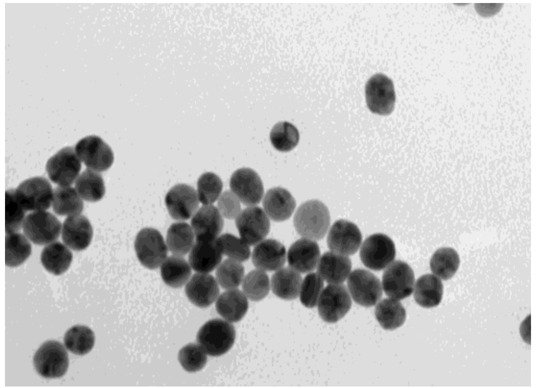



Nanomaterials





Transmission electron microscope (TEM)

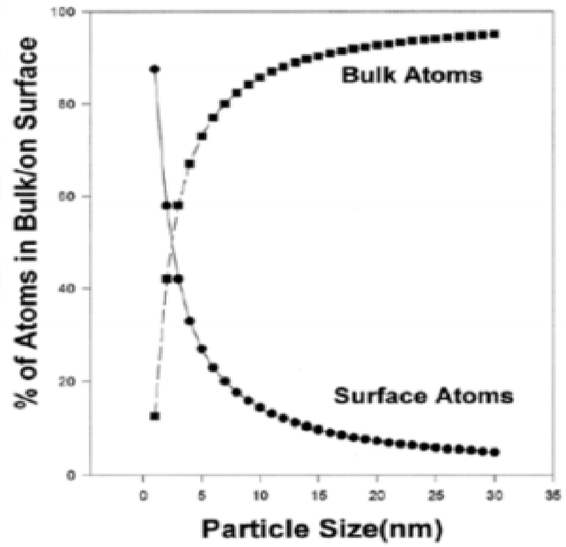


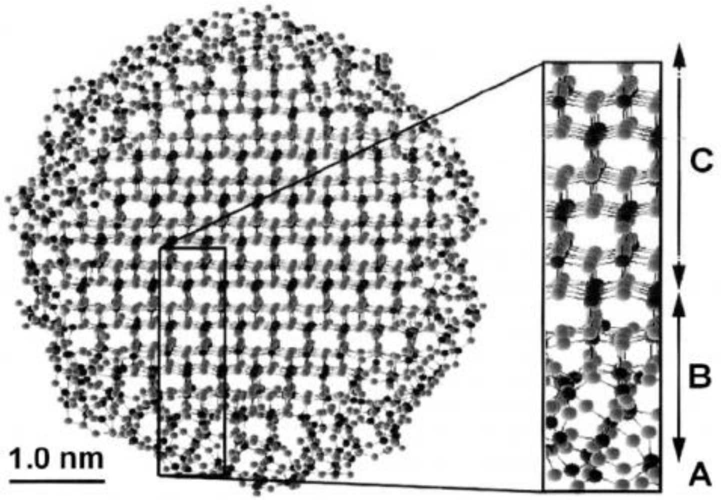
Ag nanoparticles of TEM images (Pang C, 2013)

Nanomaterials can be defined as materials possessing, at minimum, one external dimension measuring 1-100nm.

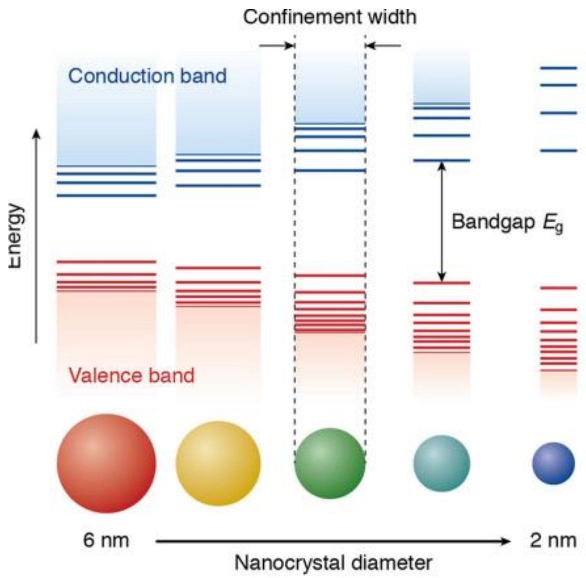
Nanomaterials

Surface effects





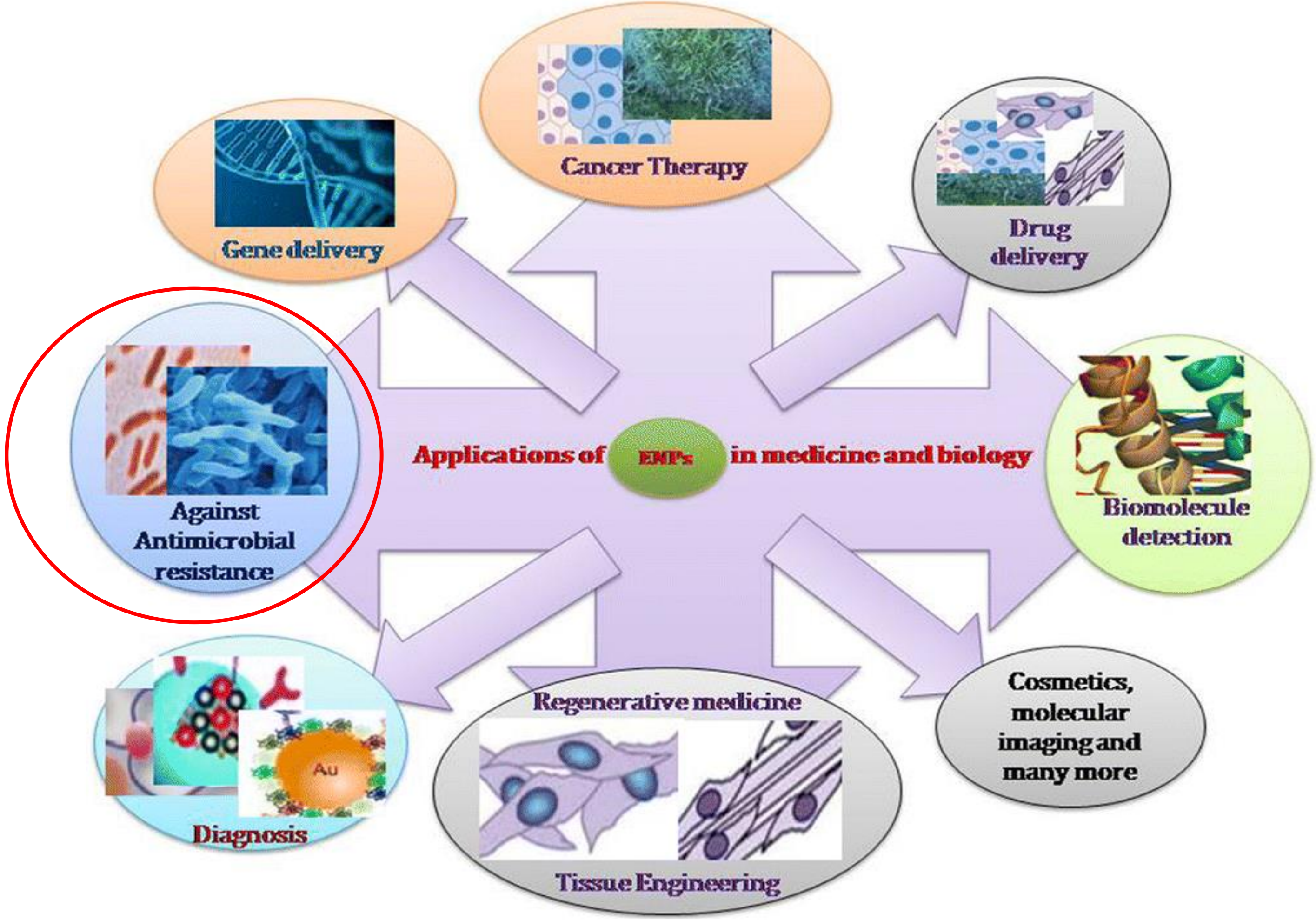
Quantum effects



Melting Point Depression: 2.5 nm Au particles 930 K, but bulk Au 1336 K.

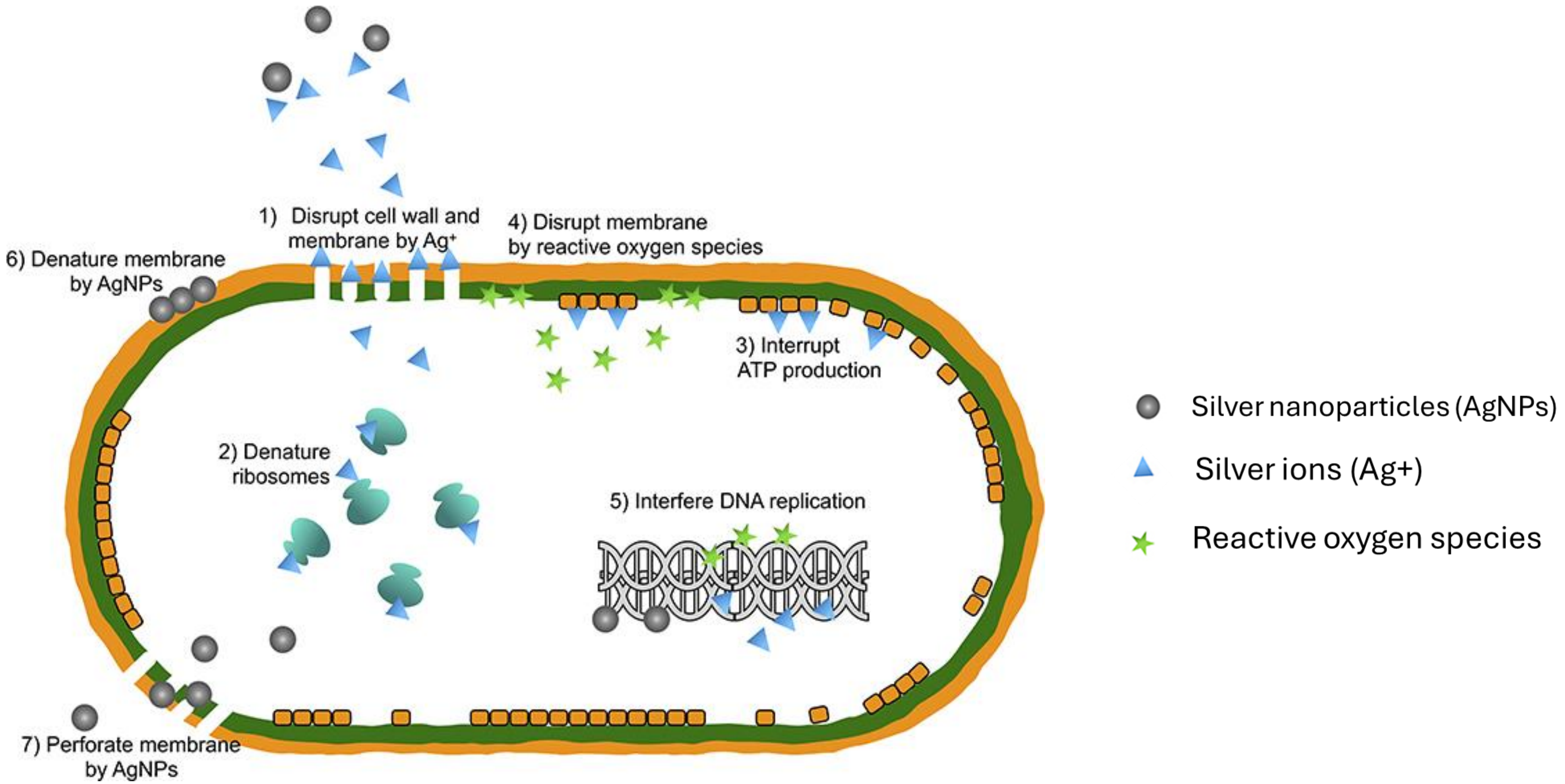
Nanomateriasl have superior properties than the bulk substances:
Mechanical strength, thermal stability, catalytic activity, electrical conductivity, magnetic properties, optical properties

Nanomaterial applications



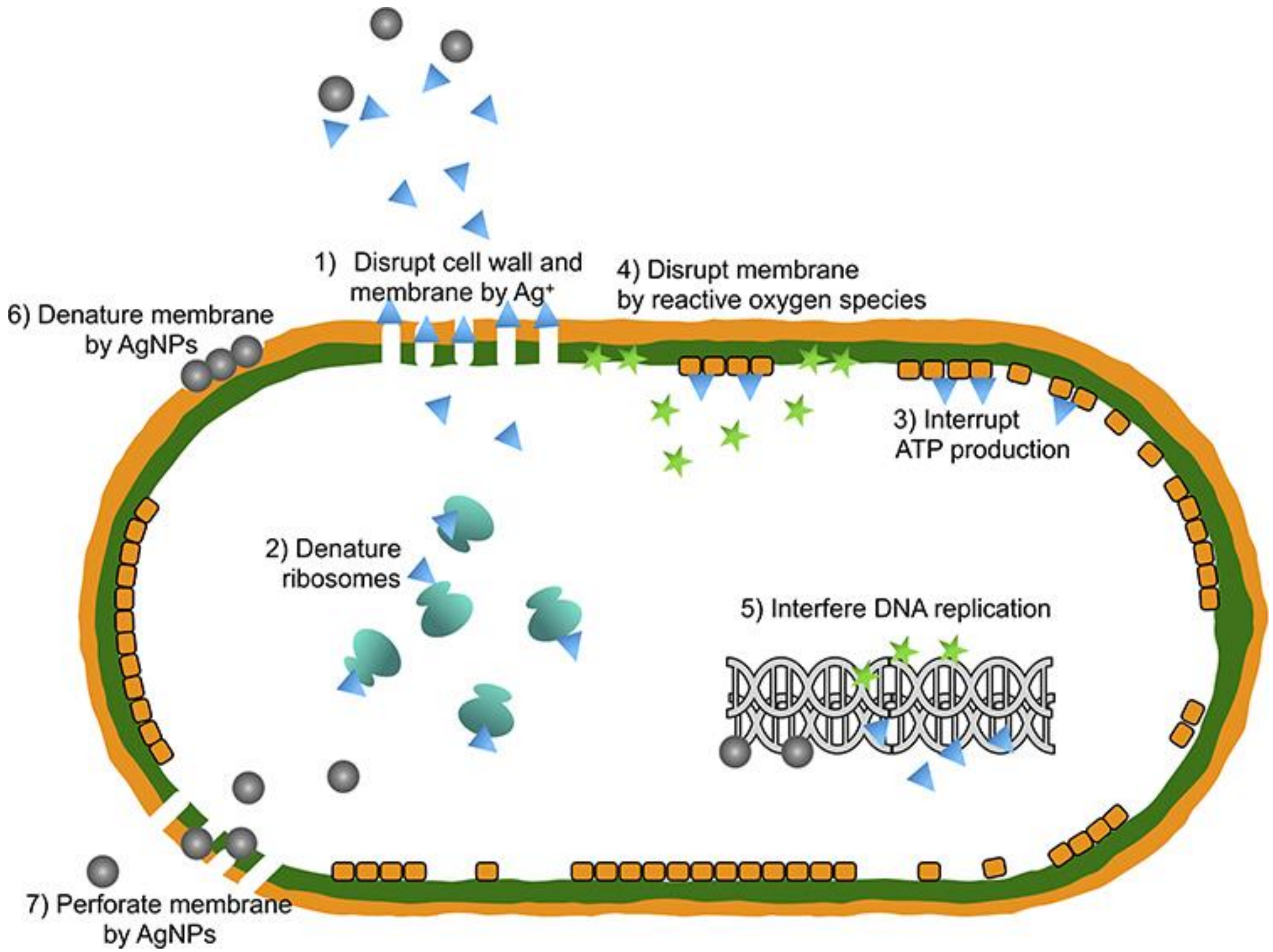
The very large surface-to-volume ratio of nanomaterials is especially useful in the medical field, which permits the bonding of cells and active ingredients

The antibacterial mechanism of silver nanoparticles



Yin, Iris Xiaoxue, et al. "The antibacterial mechanism of silver nanoparticles and its application in dentistry." International journal of nanomedicine (2020): 2555-2562.

The antibacterial mechanism of silver nanoparticles



Although bacterial resistance to antibiotics has been extensively discussed in the literature, the possible development of resistance to silver nanoparticles has not been fully explored.

Yin, Iris Xiaoxue, et al. "The antibacterial mechanism of silver nanoparticles and its application in dentistry." International journal of nanomedicine (2020): 2555-2562.

Bacterial resistance to silver nanoparticles

nature nanotechnology
ARTICLES
https://doi.org/10.1038/s41565-017-0013-y

Bacterial resistance to silver nanoparticles and how to overcome it

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Silver nanoparticles have already been successfully applied in various biomedical and antimicrobial technologies and products used in everyday life. Although bacterial resistance to antibiotics has been extensively discussed in the literature, the possible development of resistance to silver nanoparticles has not been fully explored. We report that the Gram-negative bacteria *Escherichia coli* 013, *Pseudomonas aeruginosa* CCM 3955 and *E. coli* CCM 3954 can develop resistance to silver nanoparticles after repeated exposure. The resistance stems from the production of the adhesive flagellum protein flagellin, which triggers the aggregation of the nanoparticles. This resistance evolves without any genetic changes; only phenotypic change is needed to reduce the nanoparticles' colloidal stability and thus eliminate their antibacterial activity. The resistance mechanism cannot be overcome by additional stabilization of silver nanoparticles using surfactants or polymers. It is, however, strongly suppressed by inhibiting flagellin production with pomegranate rind extract.

ARTICLES
https://doi.org/10.1038/s41565-021-00929-w
nature nanotechnology
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Role of bacterial motility in differential resistance mechanisms of silver nanoparticles and silver ions

Lisa M. Stabryla[1✉], Kathryn A. Johnston[2], Nathan A. Diemler[2], Vaughn S. Cooper[3], Jill E. Millstone[2,4,5], Sarah-Jane Haig[1,6] and Leanne M. Gilbertson[1,4✉]

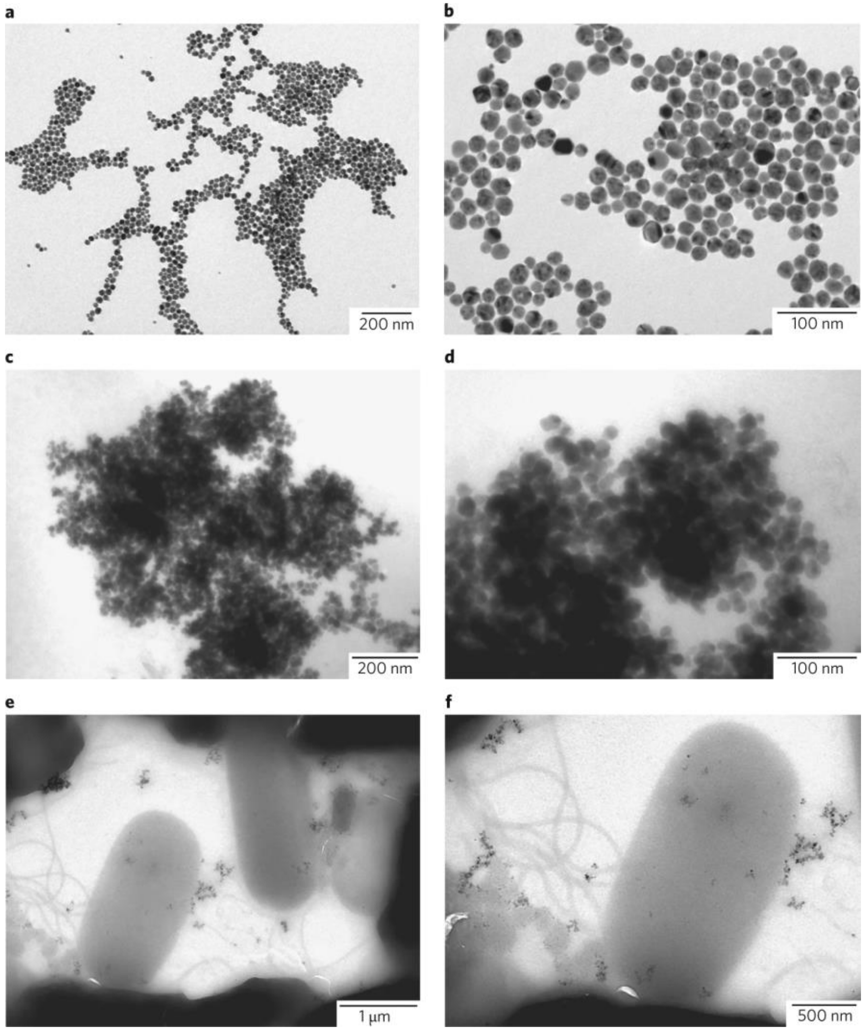
Unlike conventional antimicrobials, the study of bacterial resistance to silver nanoparticles (AgNPs) remains in its infancy and the mechanism(s) through which it evolves are limited and inconclusive. The central question remains whether bacterial resistance is driven by the AgNPs, released Ag(I) ions or a combination of these and other factors. Here, we show a specific resistance in an *Escherichia coli* K-12 MG1655 strain to subinhibitory concentrations of AgNPs, and not Ag(I) ions, as indicated by a statistically significant greater-than-twofold increase in the minimum inhibitory concentration occurring after eight repeated passages that was maintained after the AgNPs were removed and reintroduced. Whole-population genome sequencing identified a *cusS* mutation associated with the heritable resistance that possibly increased silver ion efflux. Finally, we rule out the effect of particle aggregation on resistance and suggest that the mechanism of resistance may be enhanced or mediated by flagellum-based motility.

The increasing minimum inhibitory concentration (MIC) values show the gradual development of bacterial resistance against silver NPs.

Bacteria	MIC (mg l^{-1})																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
E. coli CCM 3954	3.38	6.75	3.38	6.75	6.75	13.5	13.5	54	>54	>54	>54	>54	>54	>54	>54	>54	>54	>54	>54	>54
P. aeruginosa CCM 3955	1.69	3.38	0.89	1.69	3.38	3.38	1.69	1.69	3.38	3.38	6.75	27	54	>54	54	54	>54	54	>54	54
E. coli 013	13.5	27	13.5	27	13.5	13.5	13.5	13.5	13.5	13.5	27	27	54	>54	>54	>54	>54	54	>54	>54

Panáček, Aleš, et al. "Bacterial resistance to silver nanoparticles and how to overcome it." *Nature nanotechnology* 13.1 (2018): 65-71.

Bacterial resistance to silver nanoparticles



a,b, Pristine non-aggregated silver NPs

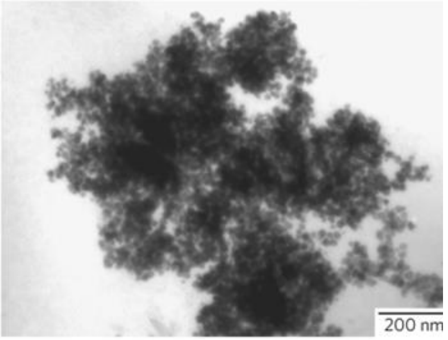
c,d, aggregated silver NPs after culturing for 24 hours with 'Ag-resistant' *Escherichia coli (E. coli) CCM 3954*.

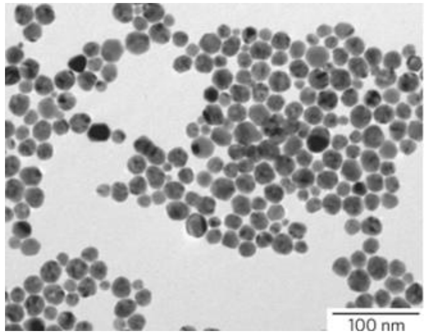
e,f, Aggregation of silver NPs induced by resistant *E. coli CCM 3954*.

The aggregation of nanosilver is the main reason for the silver nanoparticle antimicrobial resistance

Panáček, Aleš, et al. "Bacterial resistance to silver nanoparticles and how to overcome it." *Nature nanotechnology* 13.1 (2018): 65-71.

What will happen if there is
no aggregation?



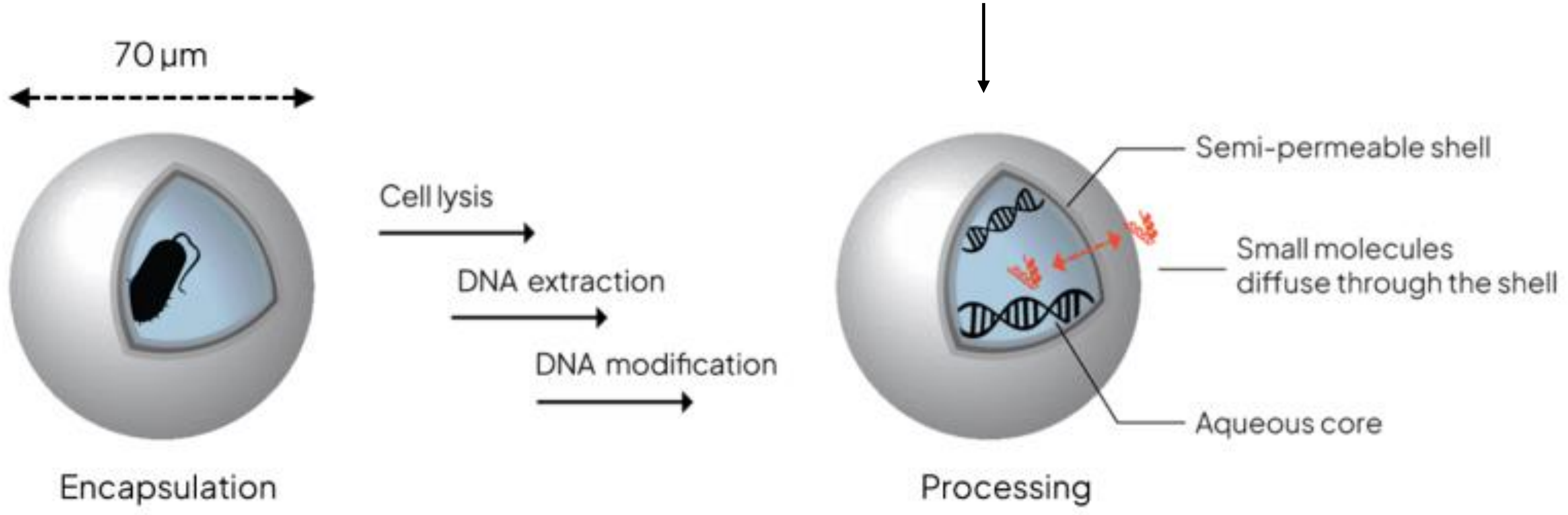


Part 1. Bacterial resistance induced by silver nanoparticles based on aggregation and no aggregation conditions

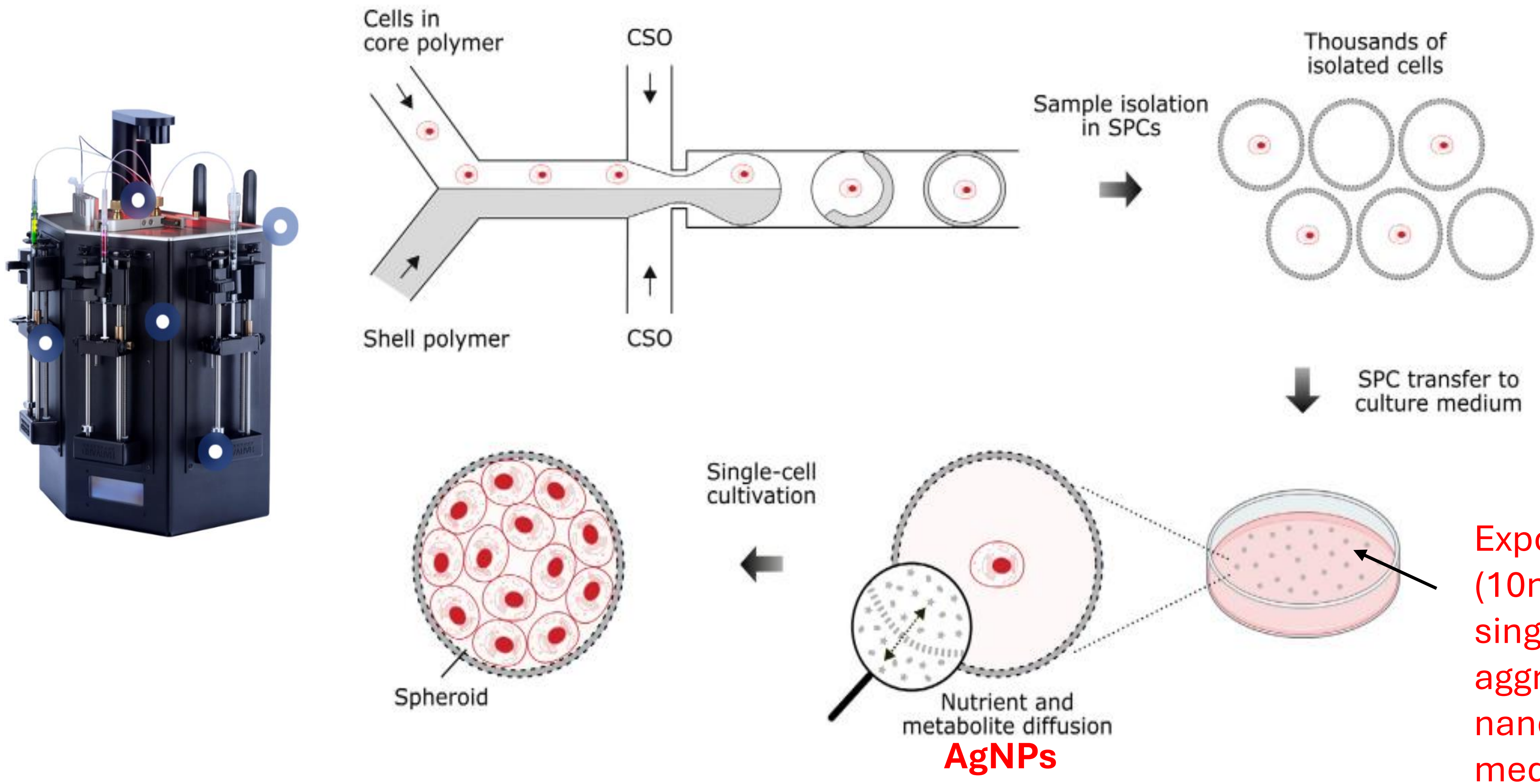
Hypothesis

Semi-permeable capsules (SPC) may be able to resolve the problem of aggregation of nanomaterials

Semi-permeable capsules (SPCs) have 30 nm holes on the shell

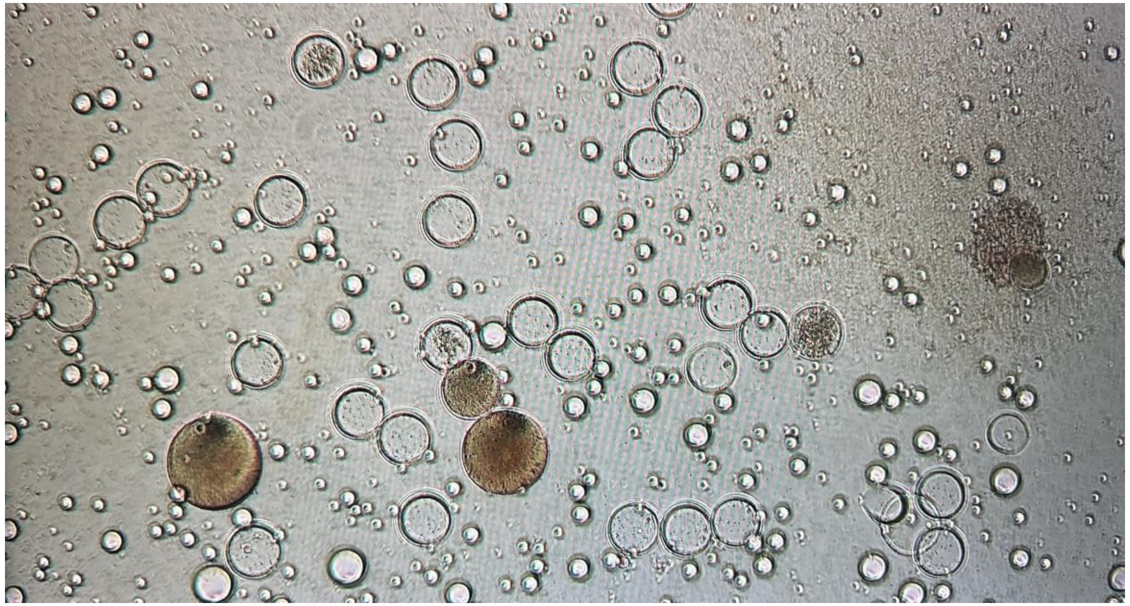


A droplet microfluidic generated cells in semi-permeable capsules (SPCs)



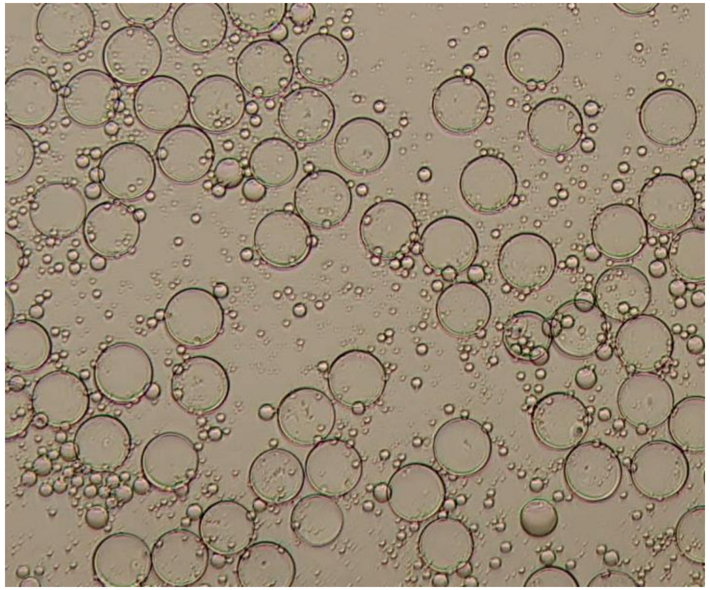
Exposure AgNPs (10nm) into SPCs single bacteria to avoid aggregation of nanoparticles in the medium.

Generating bacteria *(E.coli)* into SPCs and Monitering SPC-*E.coli* from 0h to 24hs

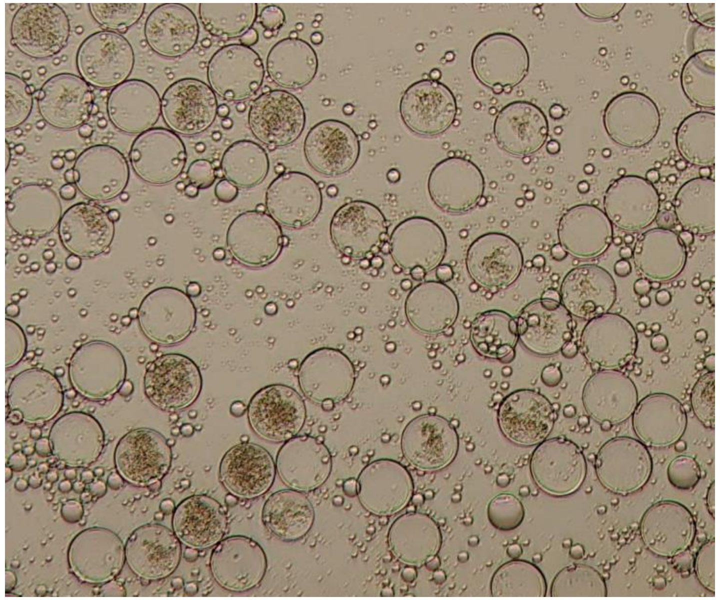


Monitering SPC-*E.coli* from 0h to 24hs

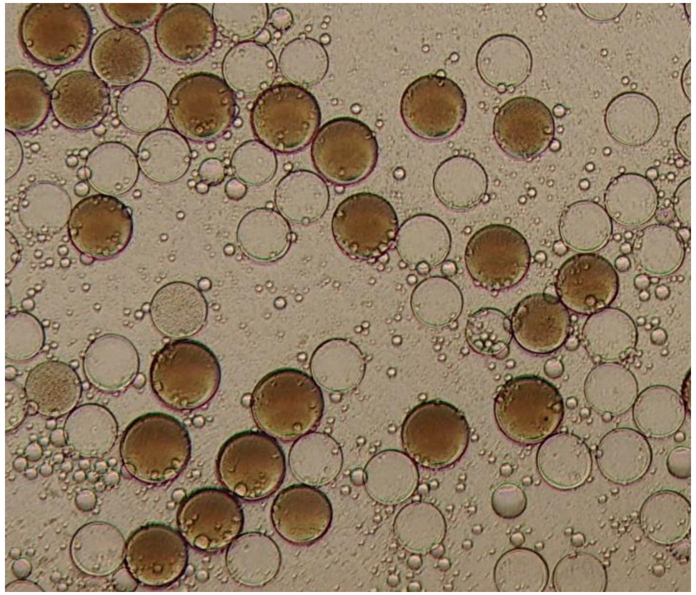
- At 3 hrs: it is able to see *E.coli*
- At 5 hrs: *E.coli* is growing in SPCs, and SPCs start growing
- At 8 hrs: SPCs stop growing



3 hrs



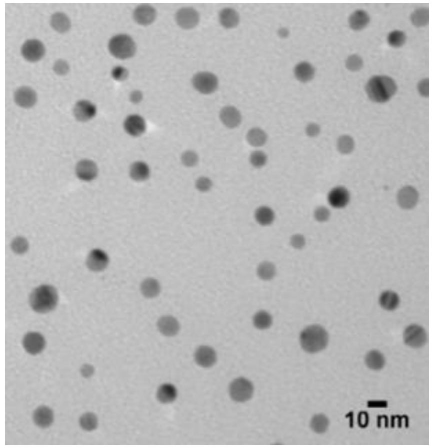
5hrs



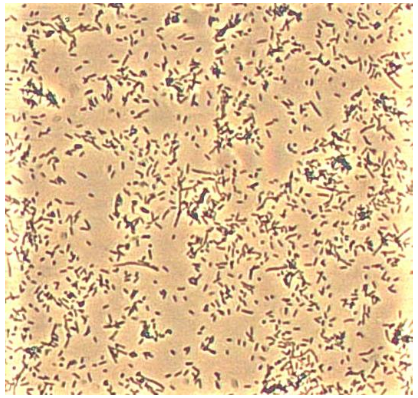
8hrs

The interactions of AgNPs with *E.coli and SPC-E.coli*

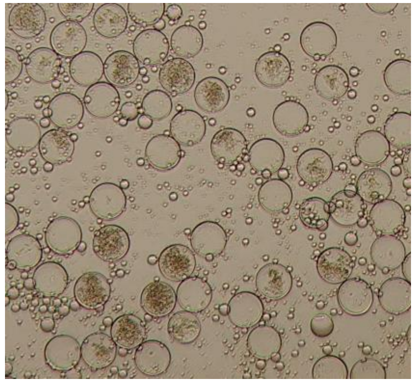
- **Nanomaterials:** 10 nm silver nanomaterials (AgNPs) (nanoCompsix, USA, OECD standard, 1mg/ml in water)
- **Bacteria:** *E. coli ATCC 25922*



Silver nanomaterials

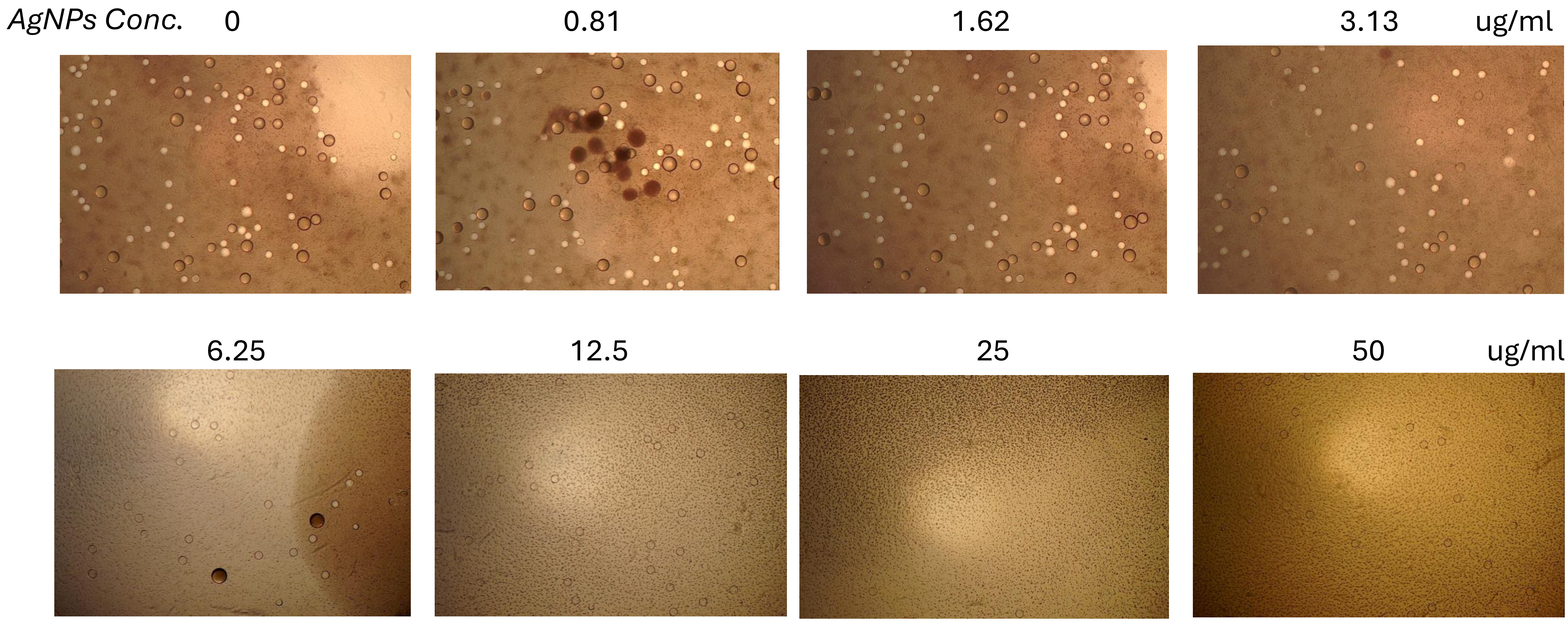


E. coli ATCC 25922



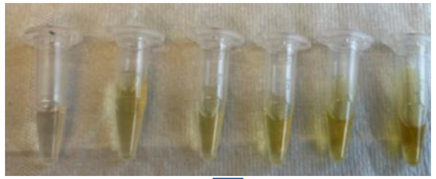
SPC-E. coli

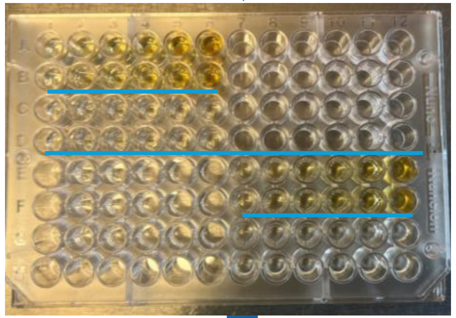
Images of SPC-*E.coli* & *AgNPs at 24hs*



MIC of AgNPs to *E.coli* and SPC-*E.coli* (27-06)

AgNPs Concentration: 0, 3.125, 6.25, 12.5, 25, 50 ug/ml



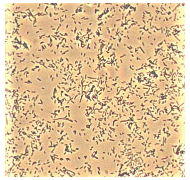


Add AgNPd at 0 hour

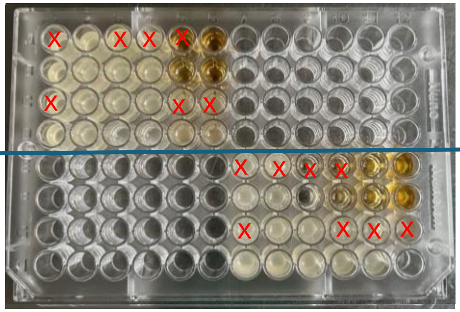
Add AgNPd at 4 hour

Add AgNPd at 0 hour

Add AgNPd at 4 hour

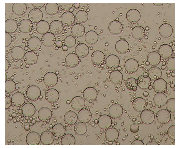


E.coli



Incubate 24 hs at 37°C

SPC E.coli



E.coli

$5x10^4$ cell/ml

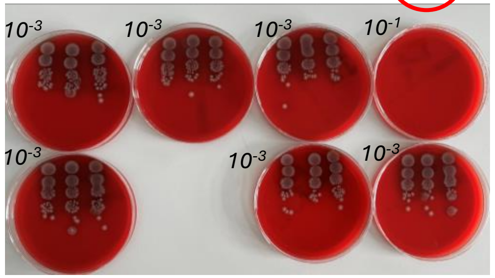
SPC E.coli

At 0h

AgNPs Concentration: 0, 6.25 12.5 25 ug/ml

0h

4h



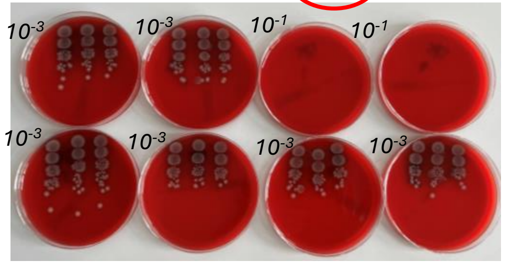
AgNPs Concentration: 0 25 50 ug/ml

At 24h

AgNPs Concentration: 0 3.125 6.25 12.5 ug/ml

0h

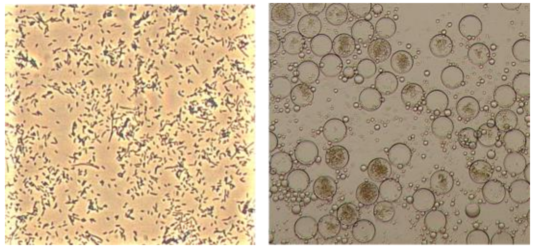
4h



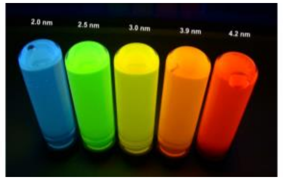
AgNPs Concentration: 0 12.5 25 50 ug/ml

Summary

1. Bacterial resistance induced by AgNPs (aggregation and no aggregation conditions)

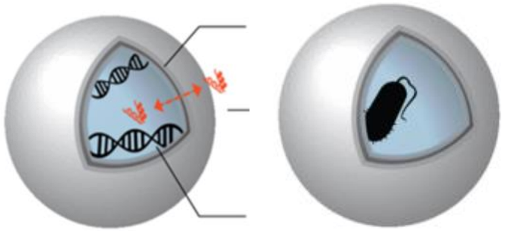


- *Dose-response issues: control the same number of initial bacteria*
- *Identify the AgNPs concentration in SPCs*

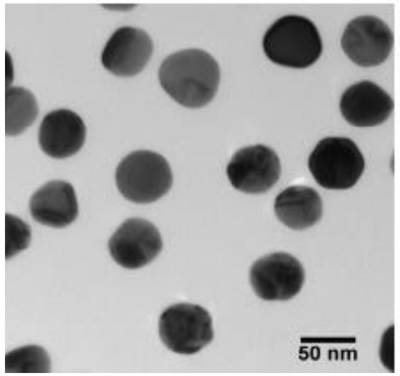


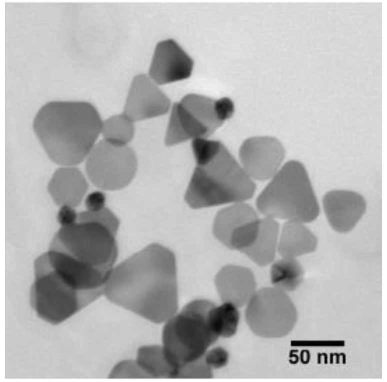
2. Visualization of nanoparticles and bacteria in SPCs: quantum dots and GFP-labeled bacteria

3. MIC and gene detection of antimicrobial nanomaterials in single-cell exposure level

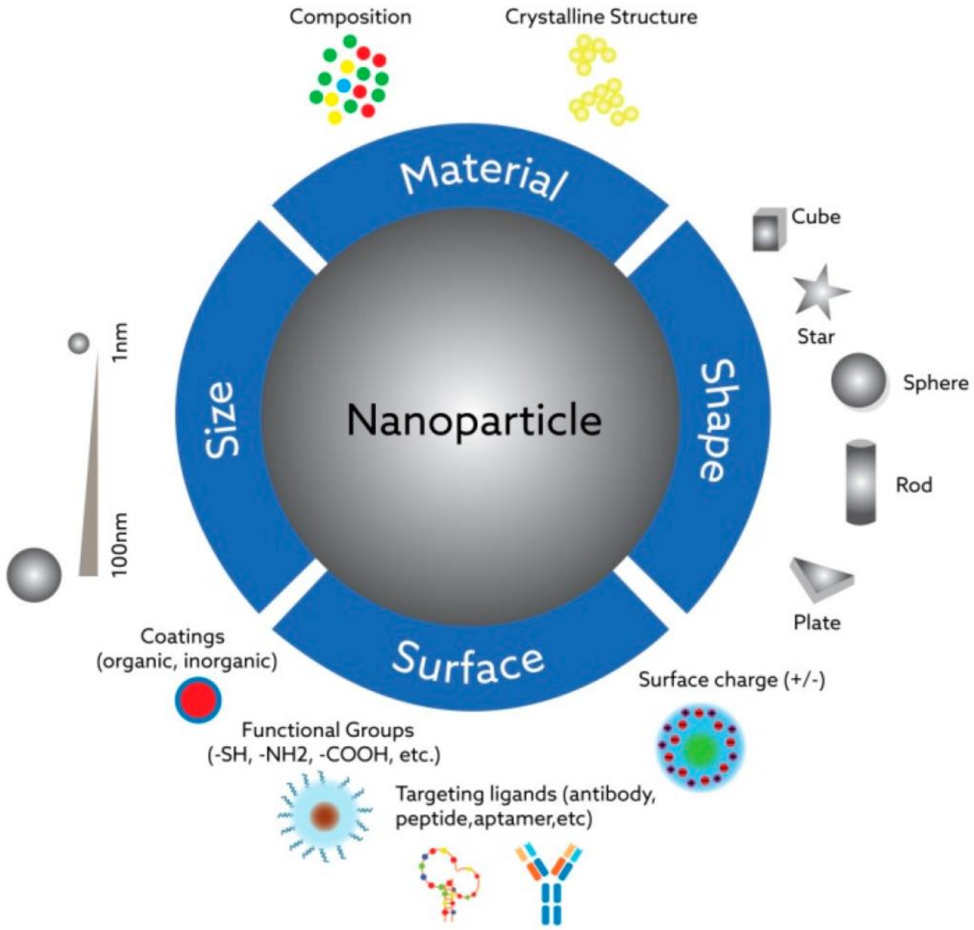


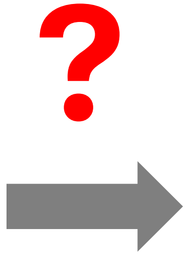
Part 2.
Bacterial resistance to silver nanomaterials based on the different shapes and size

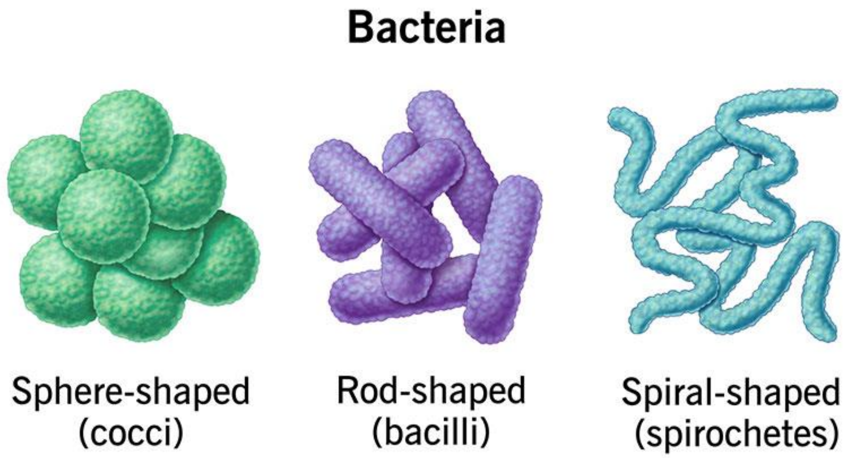




The basic features of nanomaterials support their interactions with biological systems



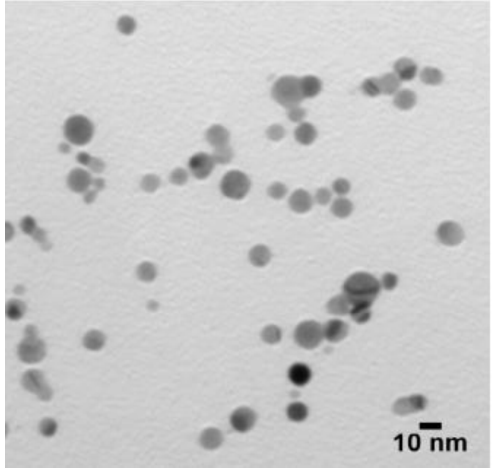




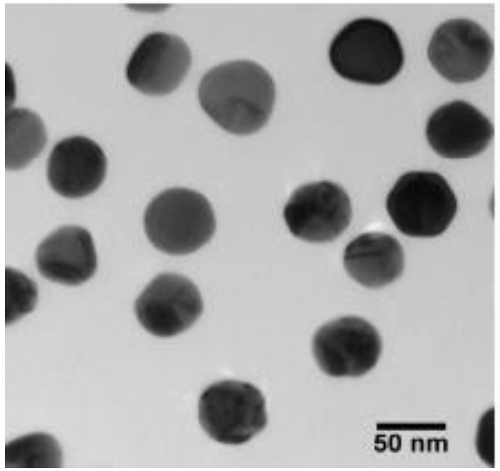
Yagublu, Vugar, et al. "Overview of physicochemical properties of nanoparticles as drug carriers for targeted cancer therapy." Journal of Functional Biomaterials 13.4 (2022): 196.

Silver nanomaterials

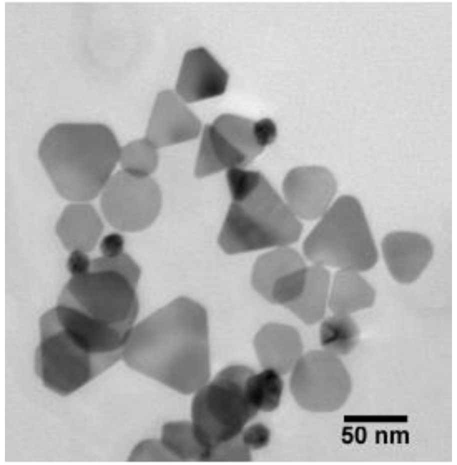
AgNanoS-10nm



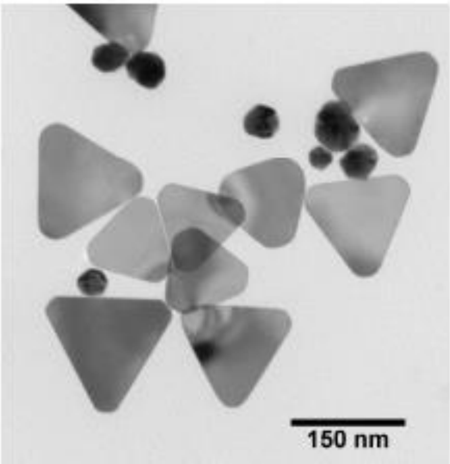
AgNanoS-50nm



AgNanoP-50nm



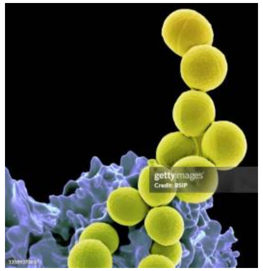
AgNanoP-100nm



Bacteria

Pseudomonas aeruginosa ATCC 27853 (P. aeruginosa ATCC 27853)

Methicillin-resistant Staphylococcus aureus (MRSA): USA 300



MIC

Size relevant

Size and shape relevant

Thank you!