POLITECNICO
MILANO 1863

Adducts of functionalized Graphene layers with Ag nanoparticles for Antimicrobial applications

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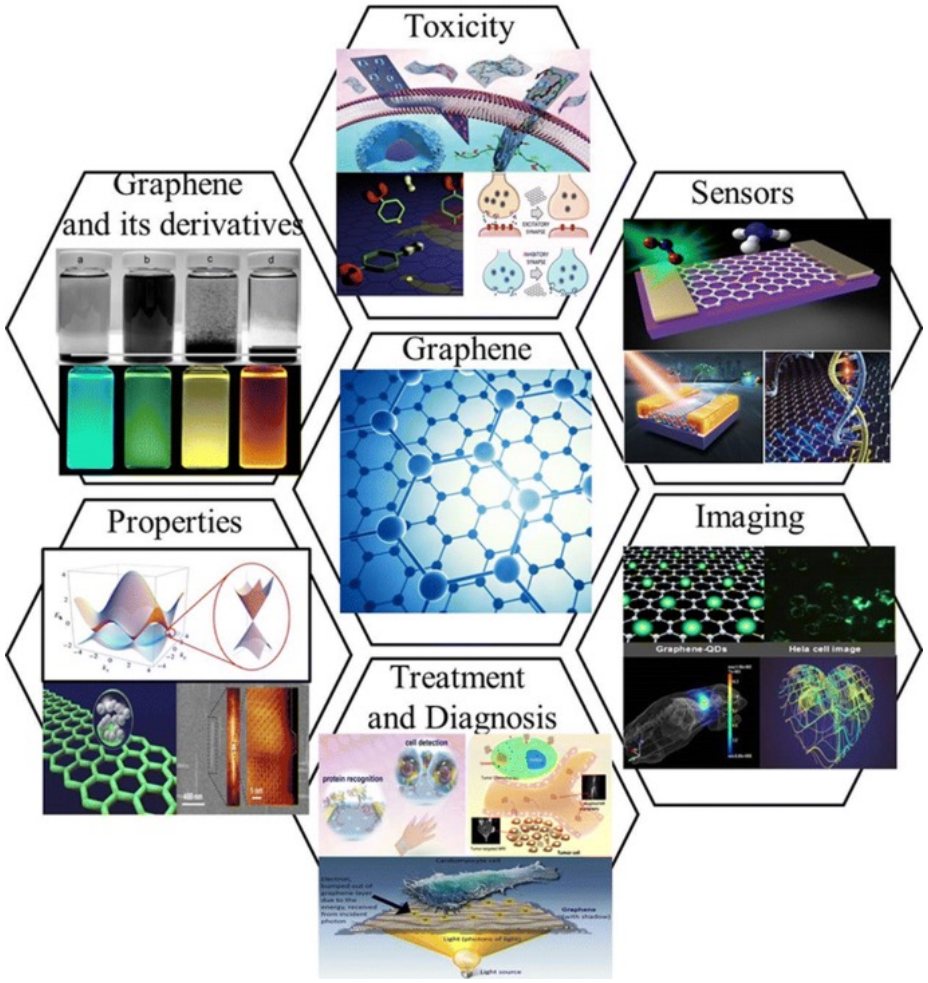
Speaker: E. Testa

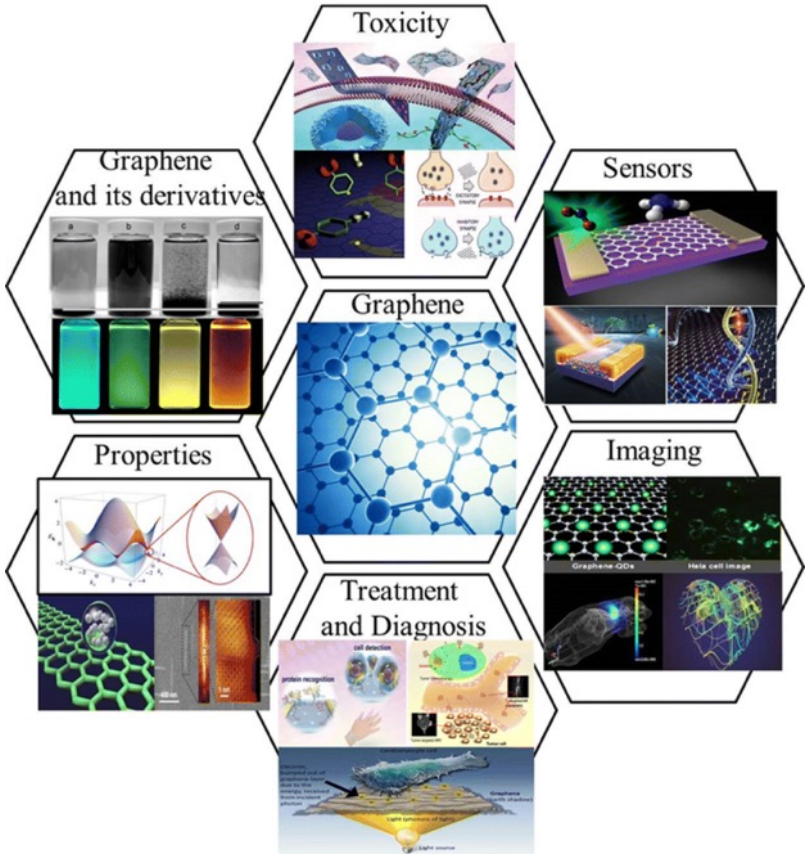
Introduction

Why Graphene ?



[Yao, J. et al., Recent advances in graphene-based nanomaterials: properties, toxicity and applications in chemistry, biology and medicine. Microchim Acta 186, 395 (2019)]

Introduction



A FUNCTIONAL SUBSTRATE

The **deposition of Ag NPs** onto graphene layers **prevents** their **natural aggregation** and accumulation.

A BIOCIDAL AGENT

Thanks to their **sharp edges**, graphene layers can act as **nanoknives** on the bacterial walls and **membranes,** leding to cell death.

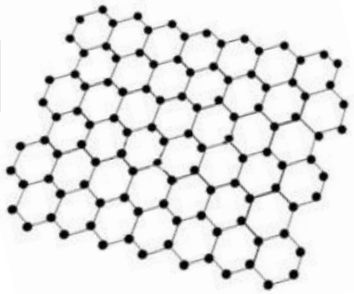
Introduction

Which type of graphene adduct?

Introduction

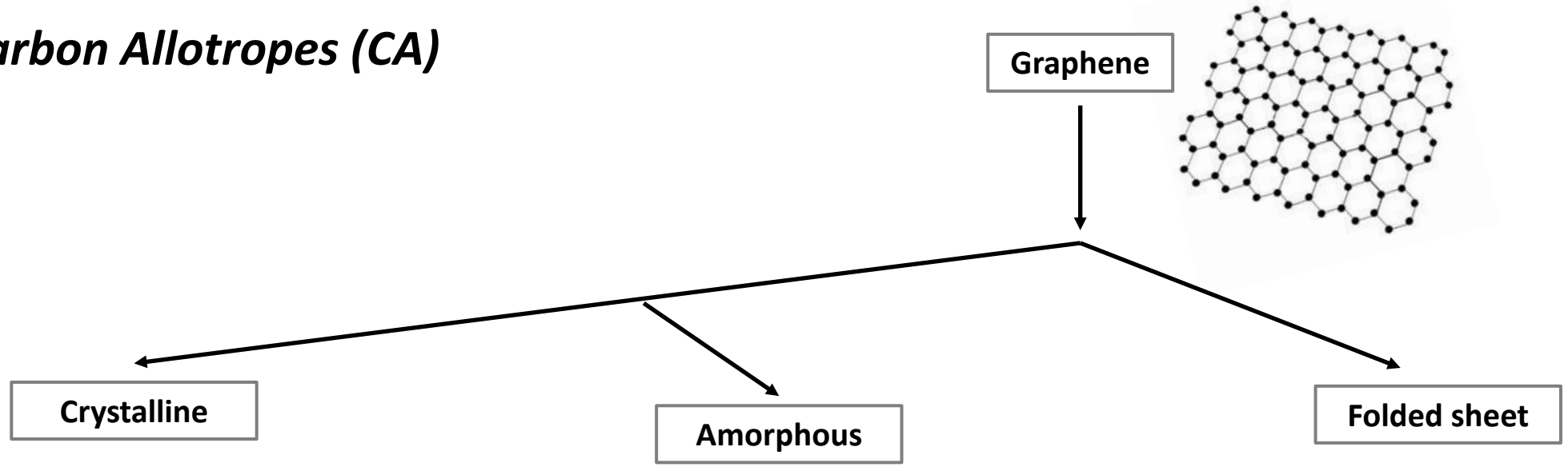
sp^2 Carbon Allotropes (CA)

Graphene



Introduction

sp² Carbon Allotropes (CA)



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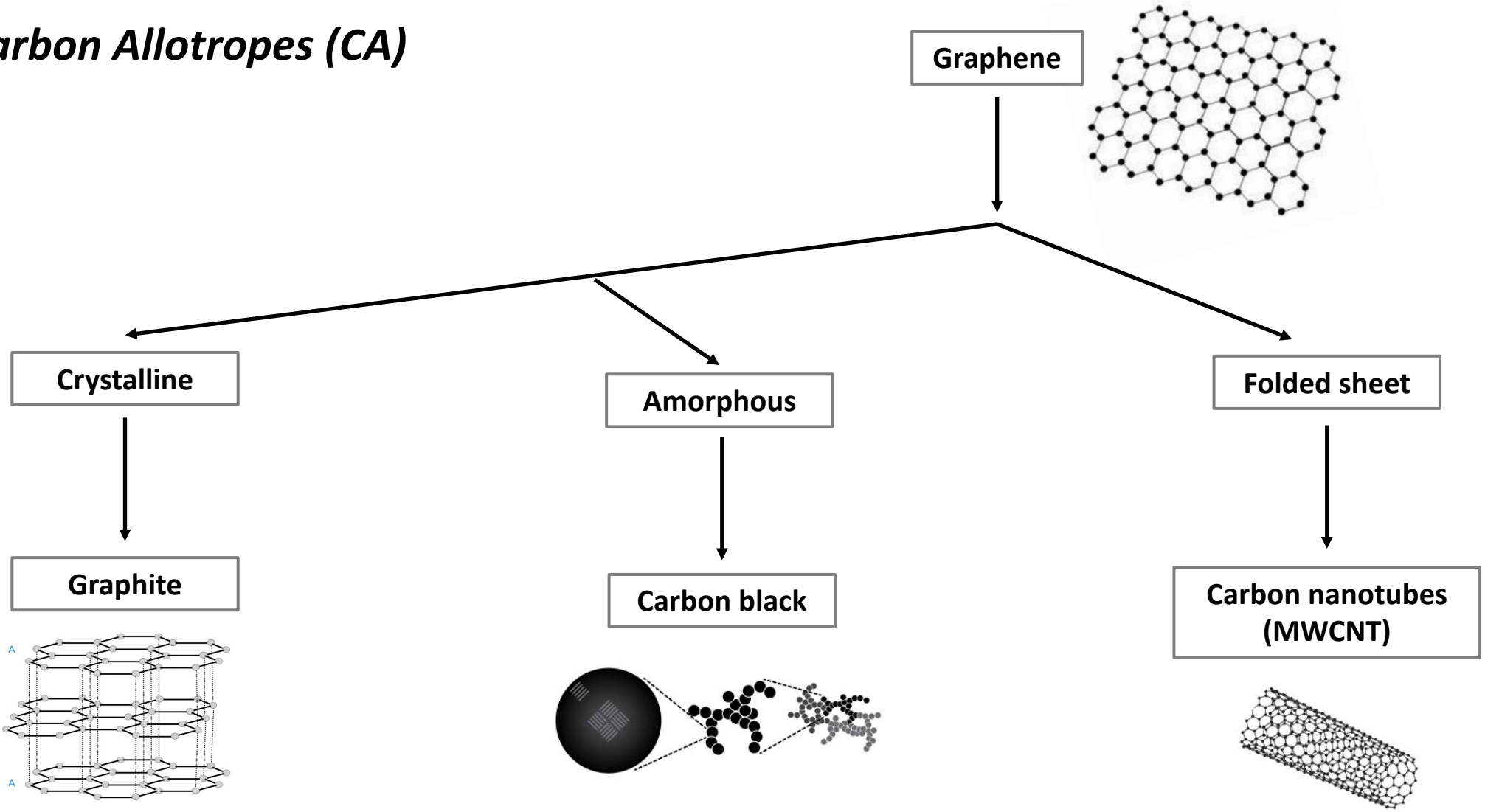
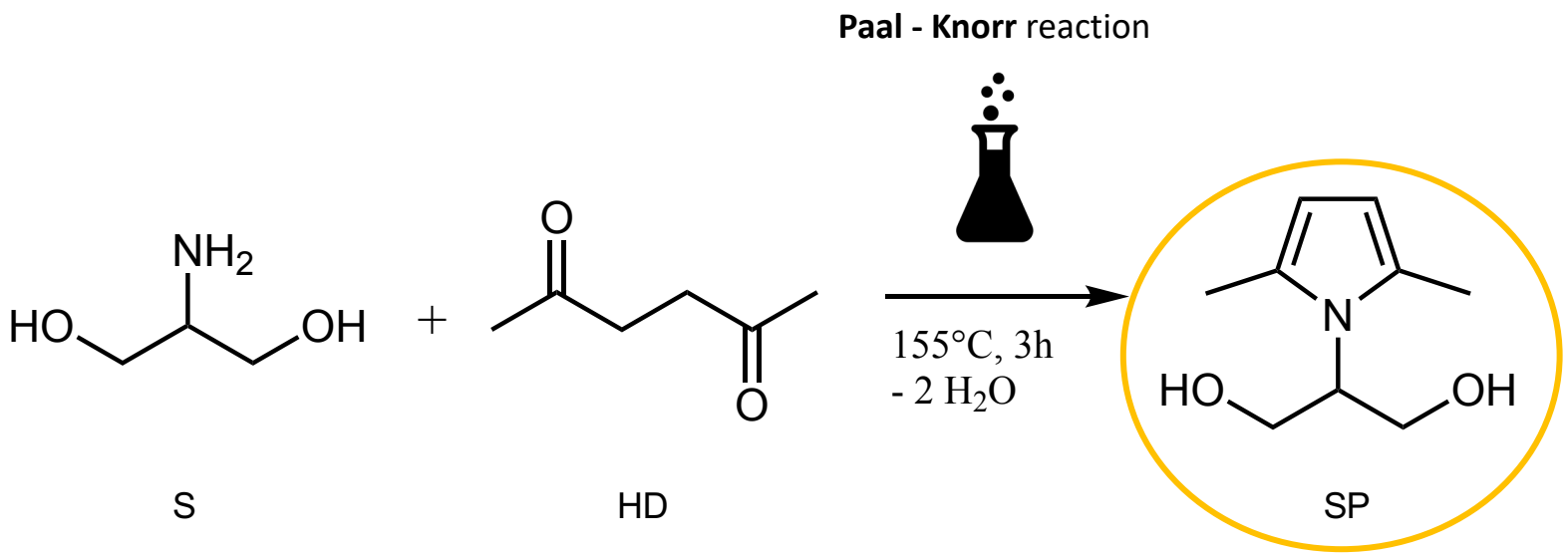


Synthesis of the adducts

How did we start?

Synthesis of the adducts: Serinol Pyrrole (SP)

Synthesis of SP



Sustainable and patented reaction mechanism

[**V. Barbera, A. Citterio, M. Galimberti**, **L. Gabriella, R. Sebastiano, S. Shisodia, A. Valerio,** «Process for the Synthesis of 2-(2,5-Dimethyl-1H-Pyrrol-1- Yl)-1,3-Propanediol and Its Substituted Derivatives», US 10,329,253 B2, **2017**]

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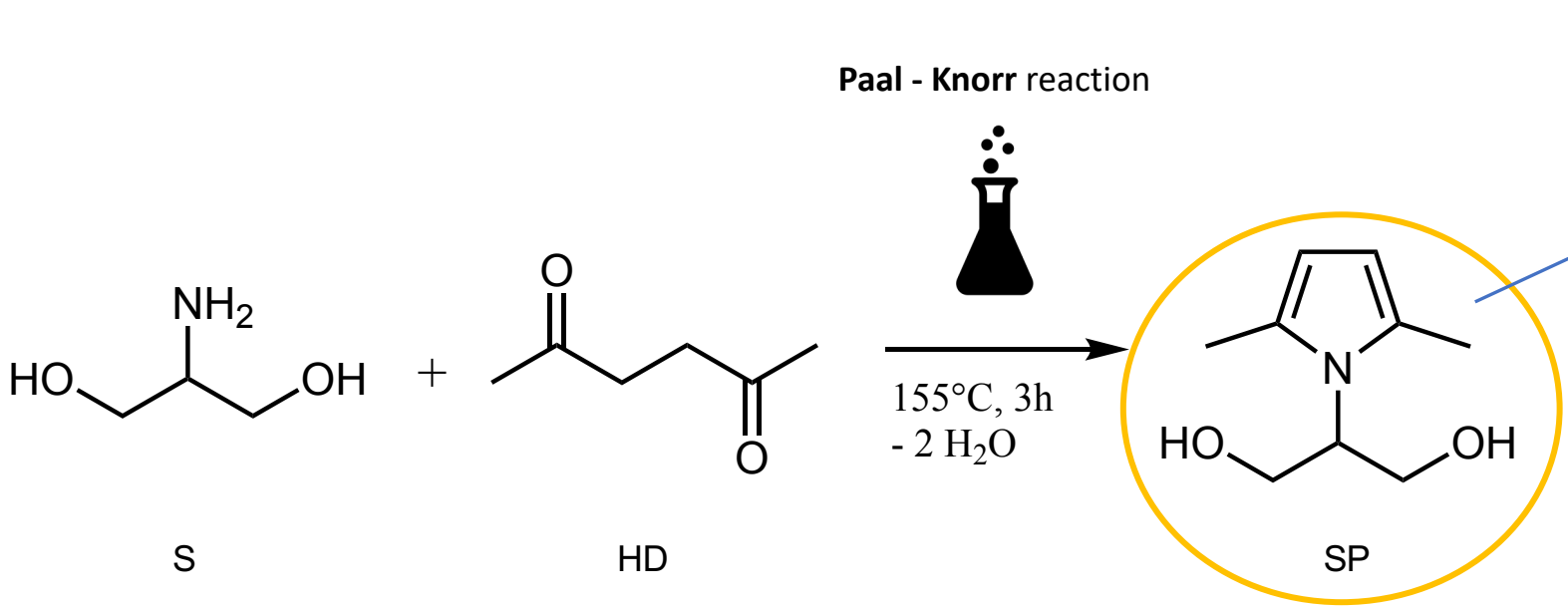
Synthesis of the adducts: Serinol Pyrrole (SP)

Synthesis of SP



Dispersability enhancer

Allows the use of **dH_2O** as solvent for a multitude of applications (e.g. **coatings**, **paintings**, ecc…)

[**V. Barbera, A. Citterio, M. Galimberti**, **L. Gabriella, R. Sebastiano, S. Shisodia, A. Valerio,** «Process for the Synthesis of 2-(2,5-Dimethyl-1H-Pyrrol-1- Yl)-1,3-Propanediol and Its Substituted Derivatives», US 10,329,253 B2, **2017**]

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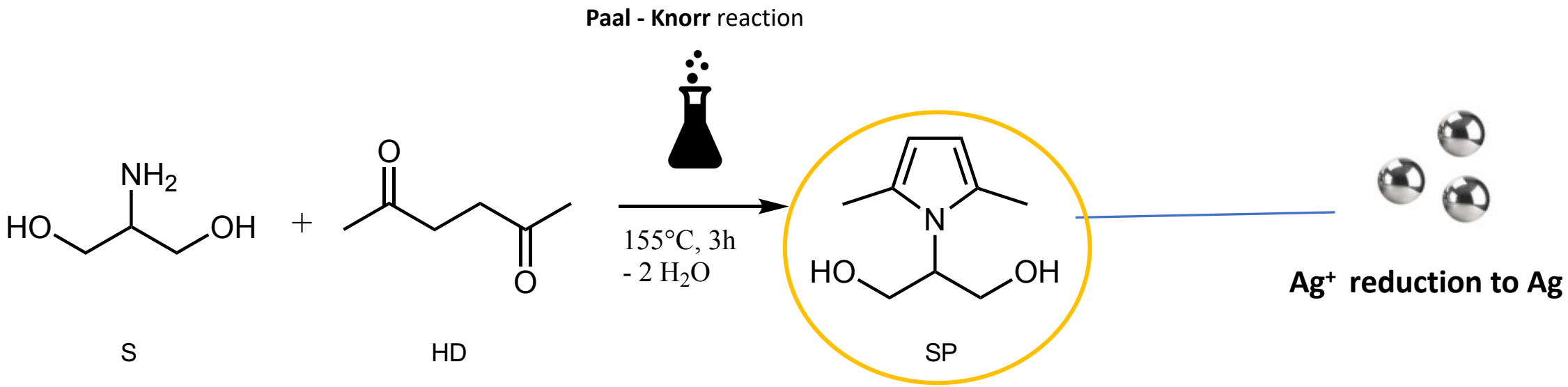

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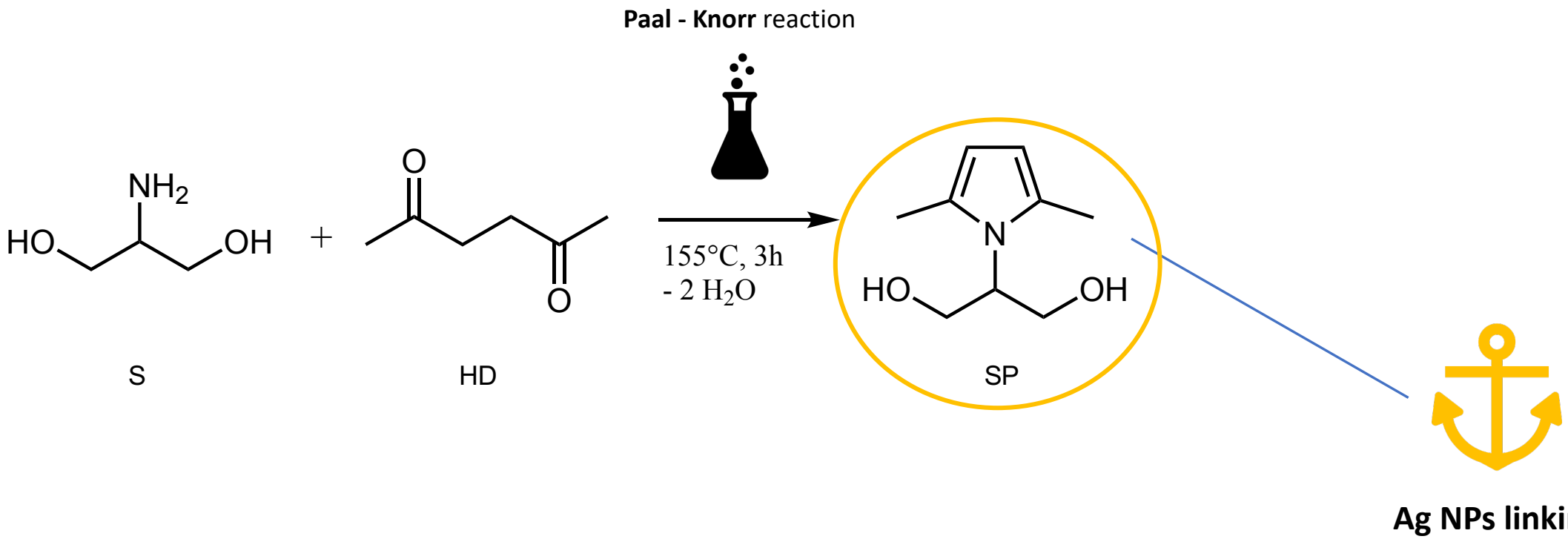

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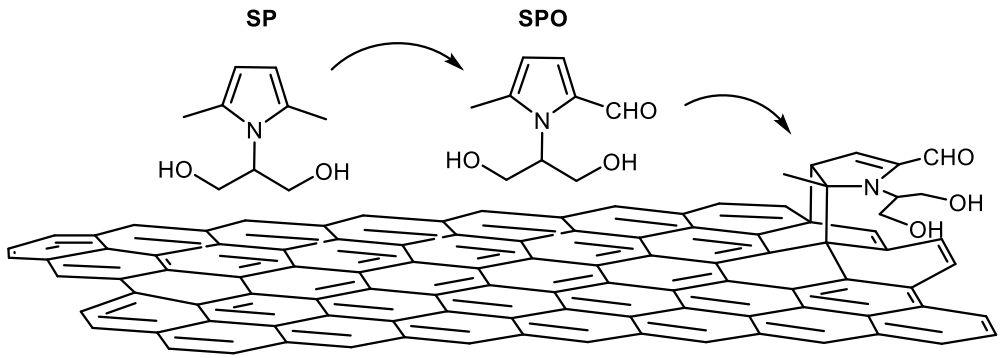
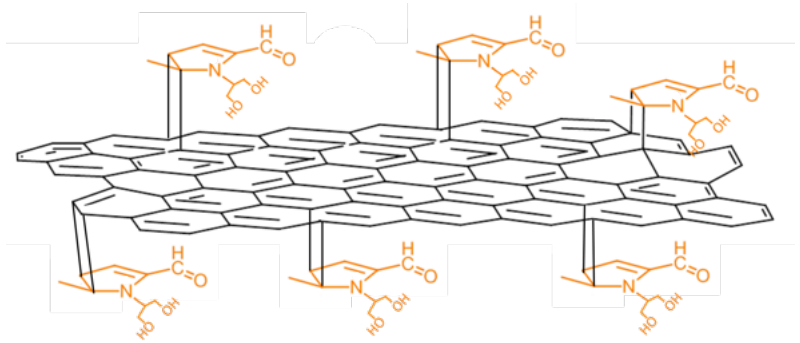
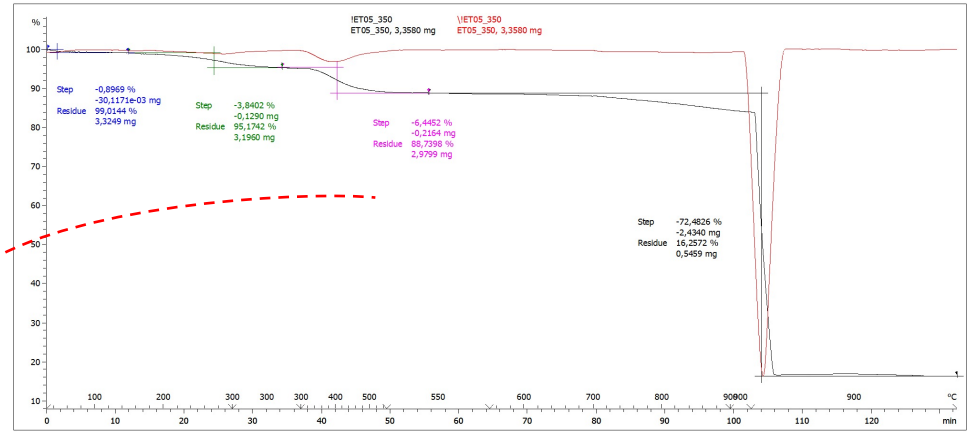
Synthesis of the adducts: substrate functionalization



Diels - Alder cycloaddition reaction mechanism



from TGA



SP content
= 10% w/w on CA

[**V. Barbera, A. Bernardi, A. Palazzolo, A. Rosengart, L. Brambilla, M. Galimberti,** Facile and Sustainable Functionalization of Graphene Layers with Pyrrole Compounds, Pure Appl. Chem., vol. 90, no. 2, pp. 253–270, **2018**]
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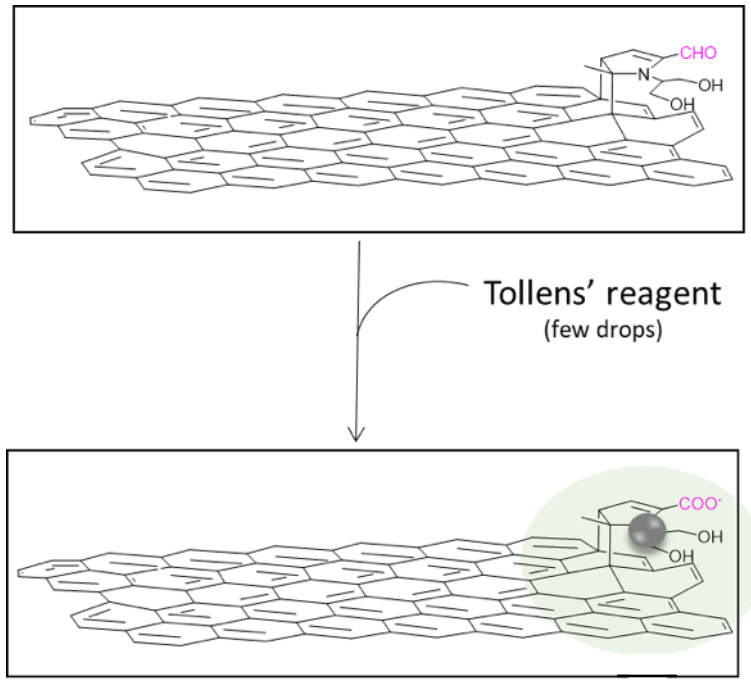
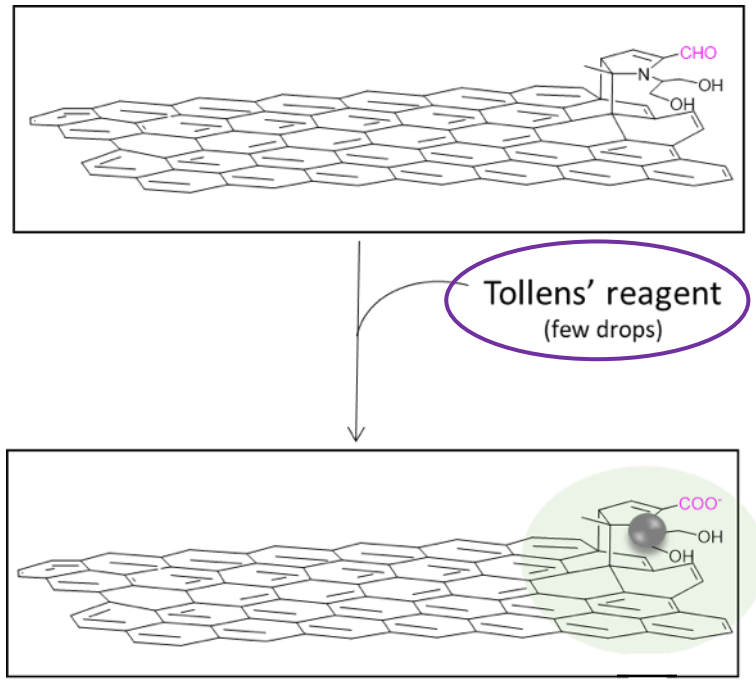
Synthesis of the adducts: decoration with Ag NPs



G-SP
H_2O suspension

G-SP/Ag NPs

Synthesis of the adducts: decoration with Ag NPs

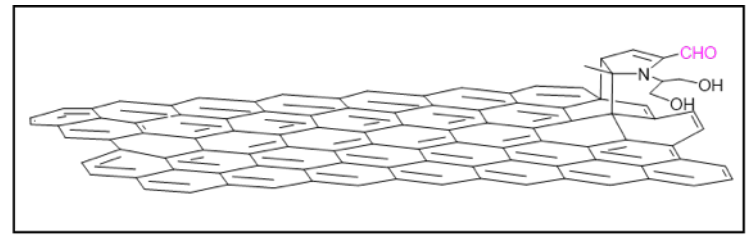


G-SP
H_2O suspension

G-SP/Ag NPs

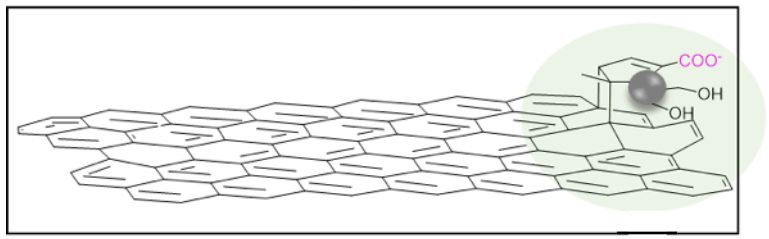
Aldehyde detection (on SP molecule) and **reduction** of Ag^+ to Ag

Synthesis of the adducts: decoration with Ag NPs



G-SP
H_2O suspension

Tollens' reagent
(few drops)



G-SP/Ag NPs

Aldehyde detection (on SP molecule) and **reduction** of Ag^+ to Ag

- Simple functionalization process
- Without reducing agents
- Sustainable process

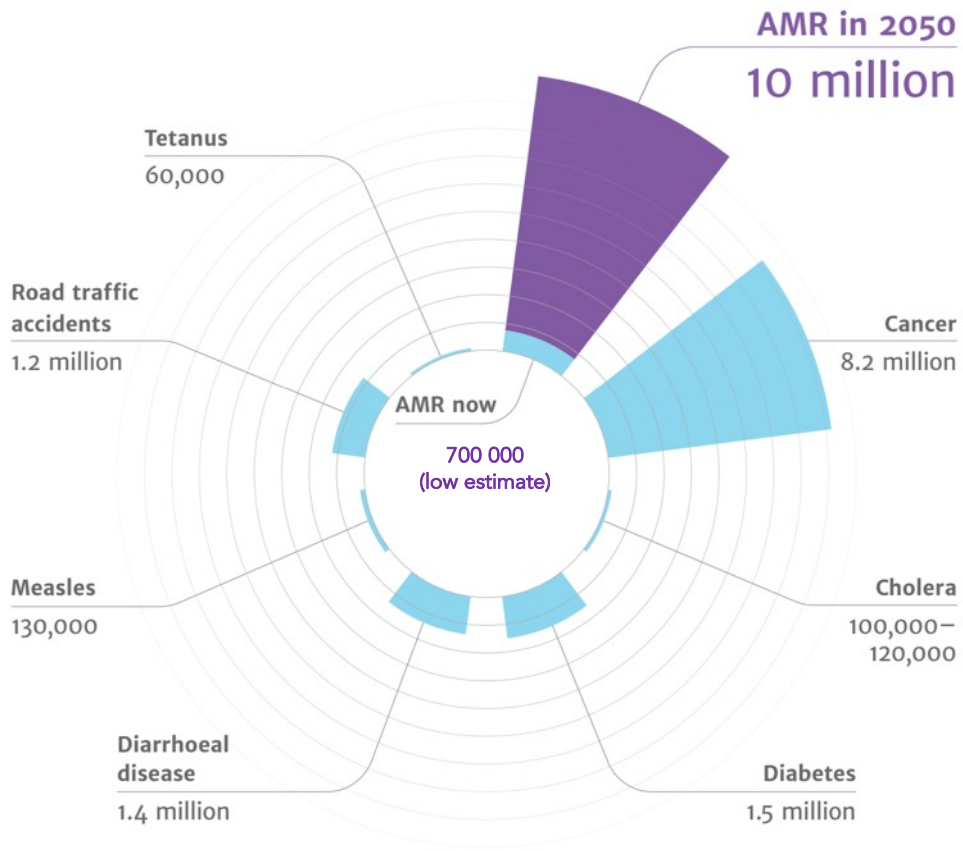
Synthesis of the adducts: decoration with Ag NPs

Why Graphene decorated with Ag NPs ?

Synthesis of the adducts: decoration with Ag NPs

Antimicrobial Resistance (AMR)

Global deaths, every year



Why Ag nanoparticles ?

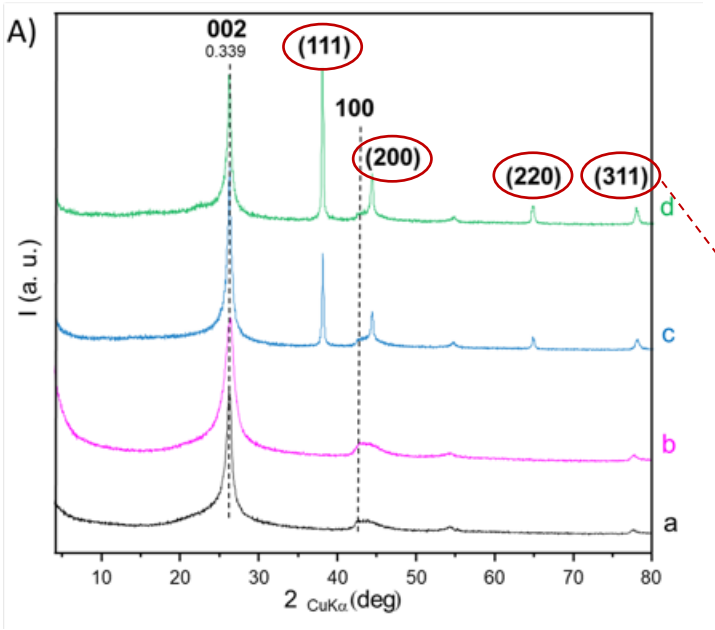
Metal NPs could be one of the best weapons to face **AMR:**

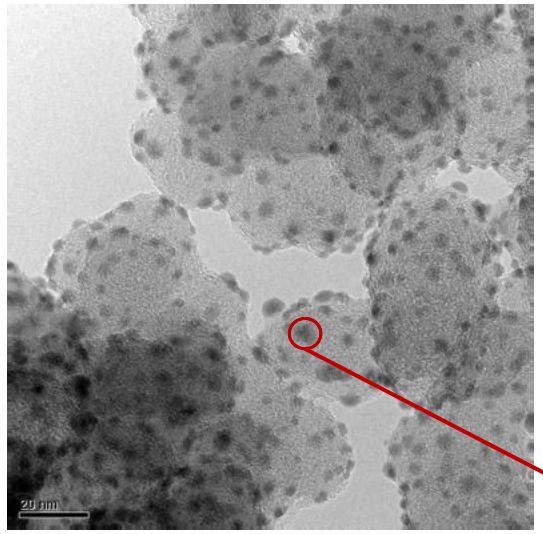
their **wide spectrum of bactericidal mechanisms** prevents bacteria to adapt and resist by means of simple mutations.

Characterization of the adducts

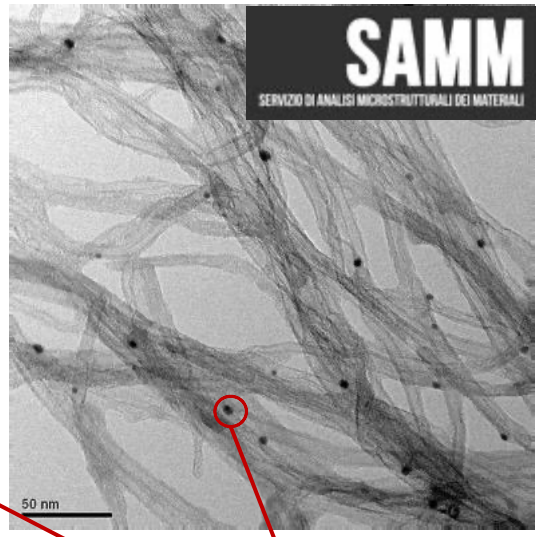
Structural and morphological analysis - HRTEM

Graphite





CB-SP/AG



CNT-SP/AG

Ag NPs: $3 - 7\ nm\ \emptyset$

- Pristine material - SP + Ag (higher loading)
- Pristine material - SP + Ag
- Pristine material - SP
- Pristine material

Facets characteristic of Ag NP cristallographic structure

Biological assay

Biological assay

Test: ASTM Standard Guide E2315 – 16

Bacteria: E. coli JM109 – DSM3423

Material state: Dispersions of powders

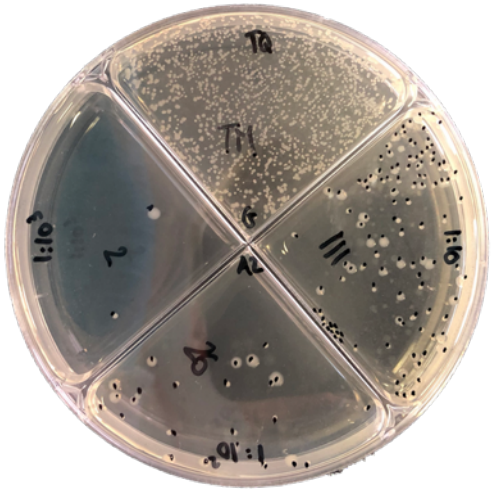
Inoculum concentration: ~10^6 CFU/mL

Culture medium: LB / PBS 1:100 (v/v)

Culture conditions: 24h, 37°C, 5% CO_2, 90% R.H.

Dynamic conditions

μBioMI LAB



Test: ISO 10993-5, 8.3 – Direct contact evaluation

Cells: MCF-7, Human epithelial fibroblasts

Inoculum concentration: ~10^4 cells/well, 31250 cells/cm^2

Material state: Dispersions of powders

Culture medium: 10% PBS + 90% cEMEM

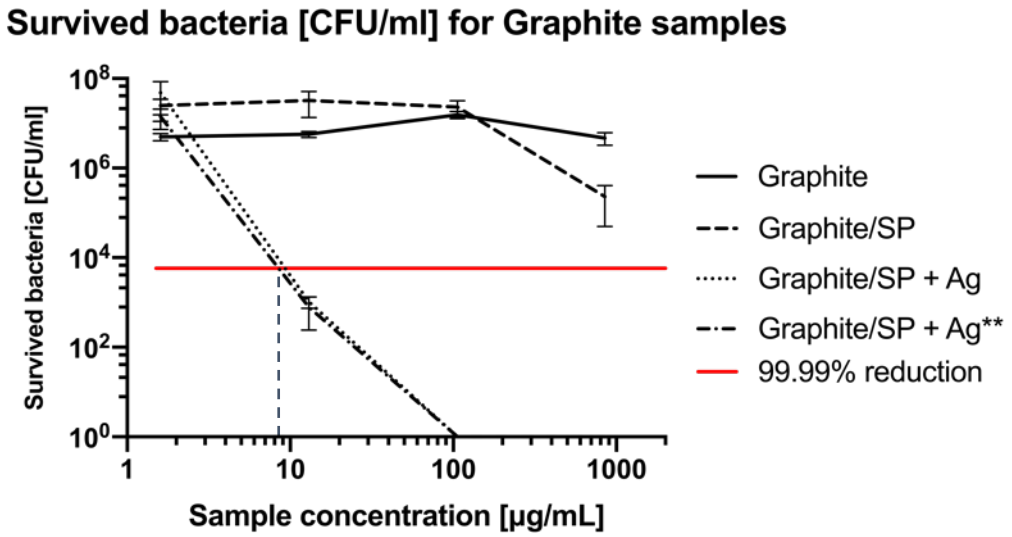
Culture conditions: 24h, 37°C, 90% R.H.

Viability evaluation: Neubauer chamber, trypan blue

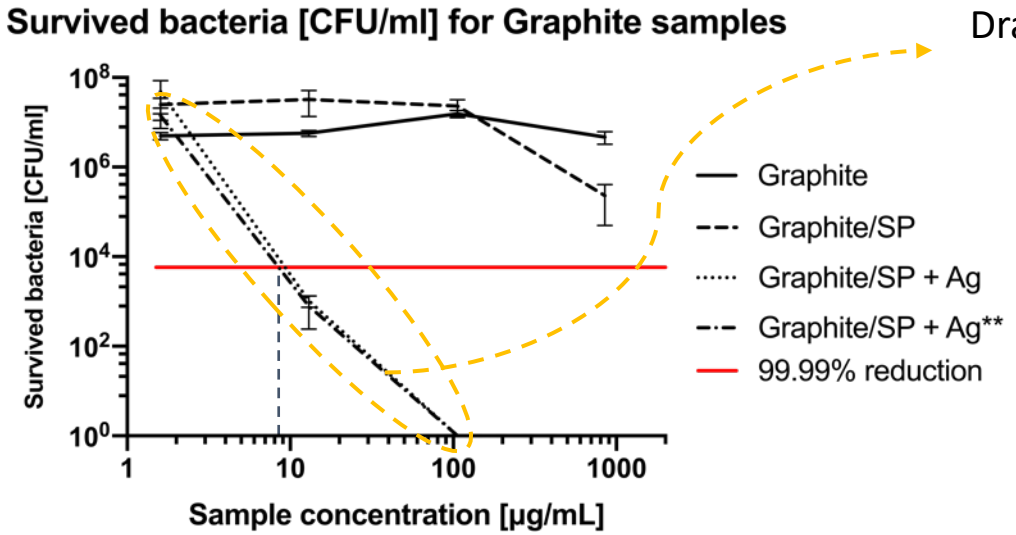
BioCell

Laboratory of Biocompatibility and *Cell Culture*

Antimicrobial activity: Results

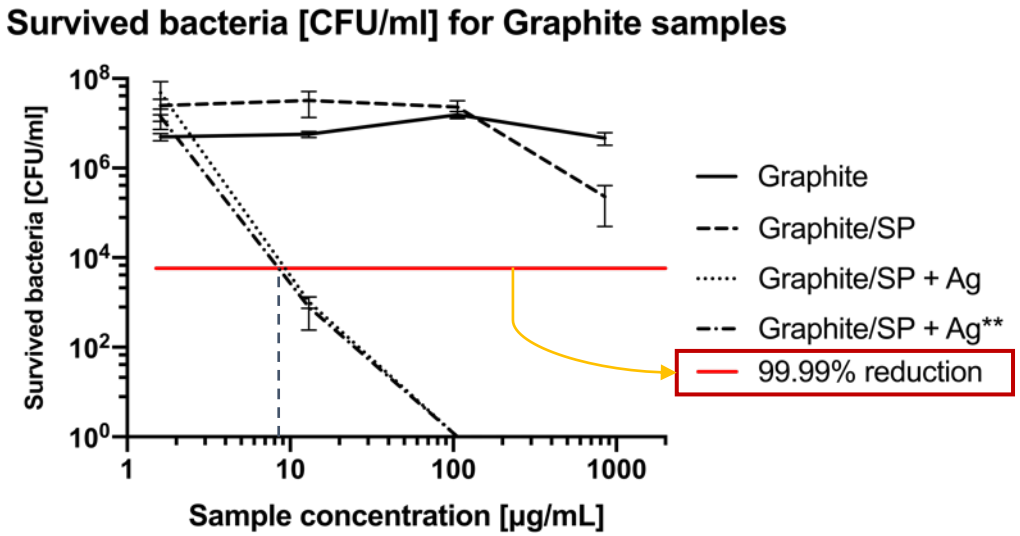


Antimicrobial activity: Results



Drastic decrease of bacteria viability for both **Ag-loaded samples!**

Antimicrobial activity: Results



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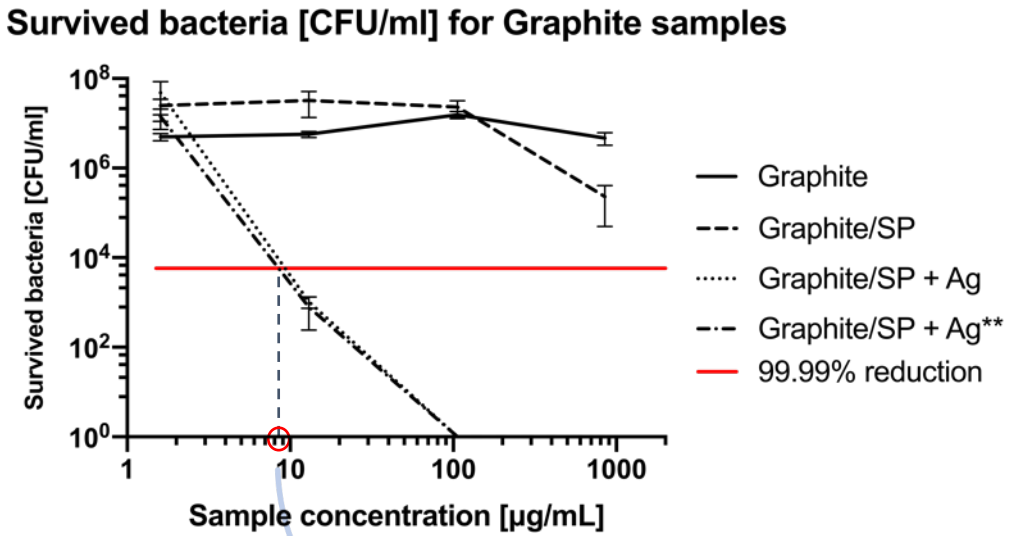
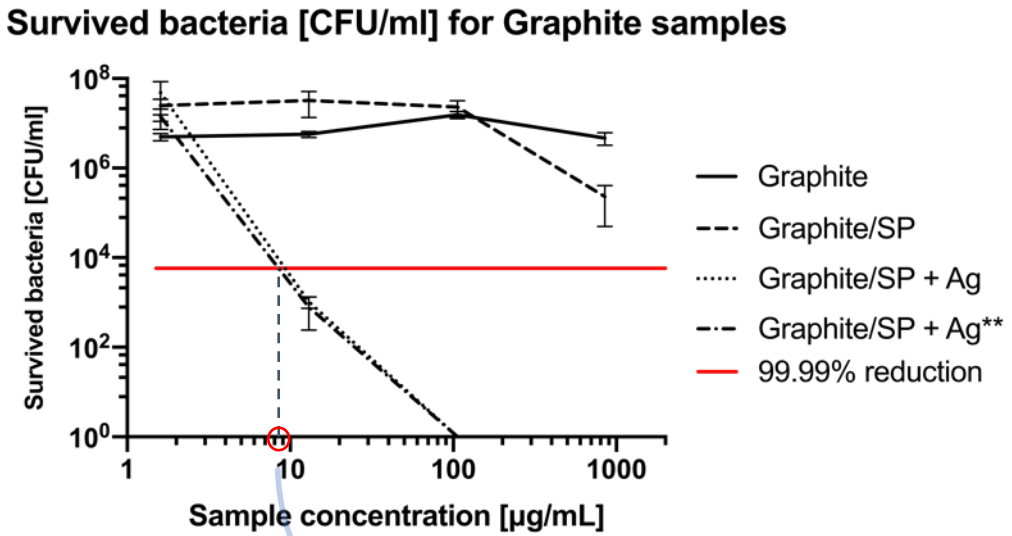


MBC

(Minimal bactericidal concentration (99.99% killing)

Antimicrobial activity: Results



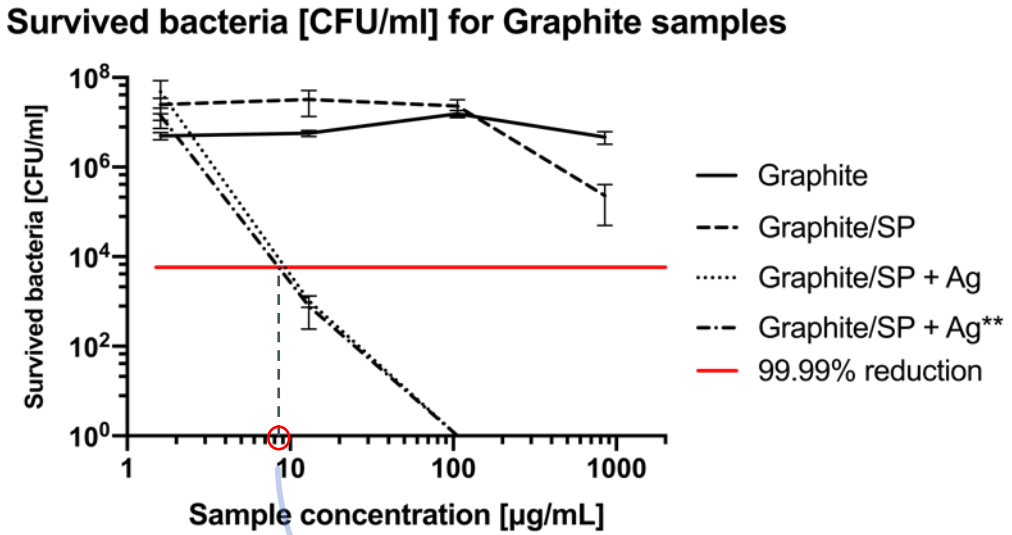
MBC

(Minimal bactericidal concentration (99.99% killing)

Sample	MBC [µg/mL - ppm] [a]
Graphite - SP/Ag	10
Graphite - SP/Ag**	9
CB - SP/Ag	195
CB - SP/Ag**	180
CNT - SP/Ag	12
CNT - SP/Ag**	7

a: Concentration at 99.99% bacterial reduction

Antimicrobial activity: Results



MBC

(Minimal bactericidal concentration (99.99% killing)

The bactericidal activity is **allotrope dependent**!

Sample	MBC [µg/mL - ppm] [a]
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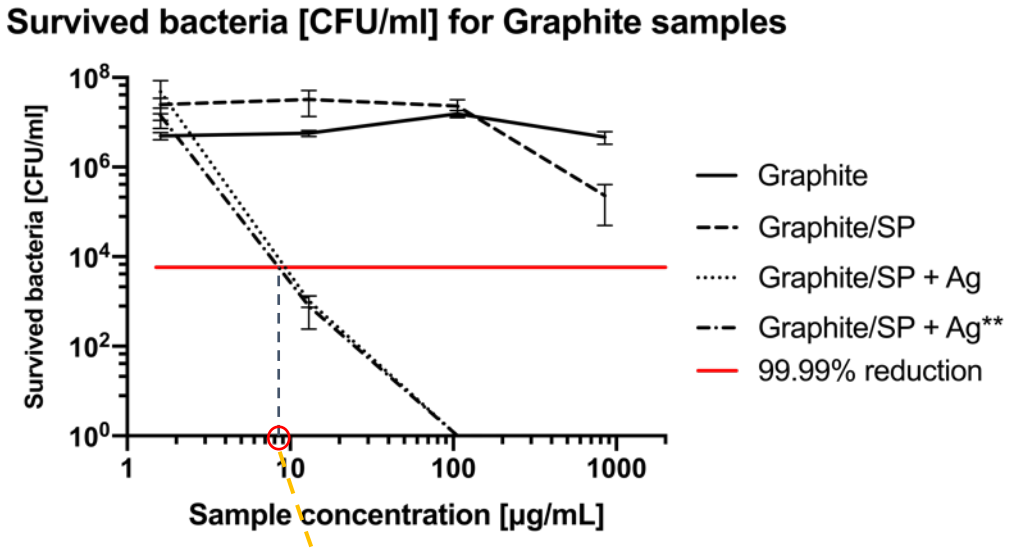
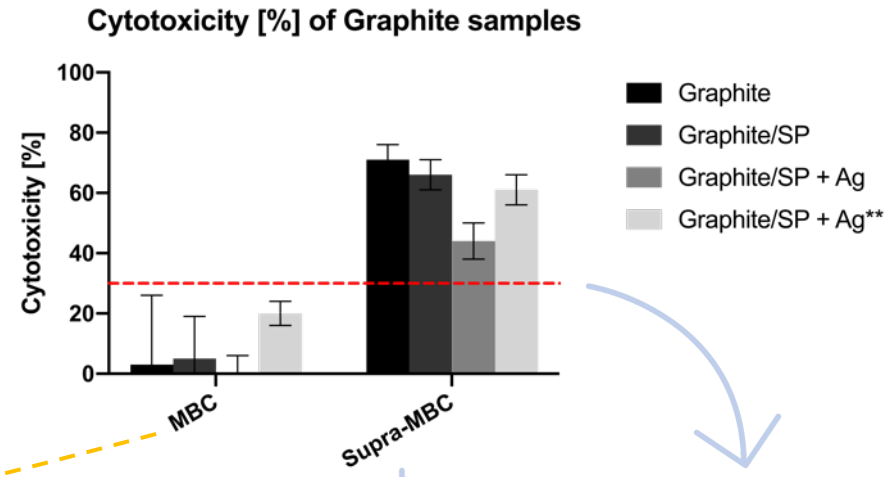
a: Concentration at 99.99% bacterial reduction

Antimicrobial activity vs. cytotoxicity of materials

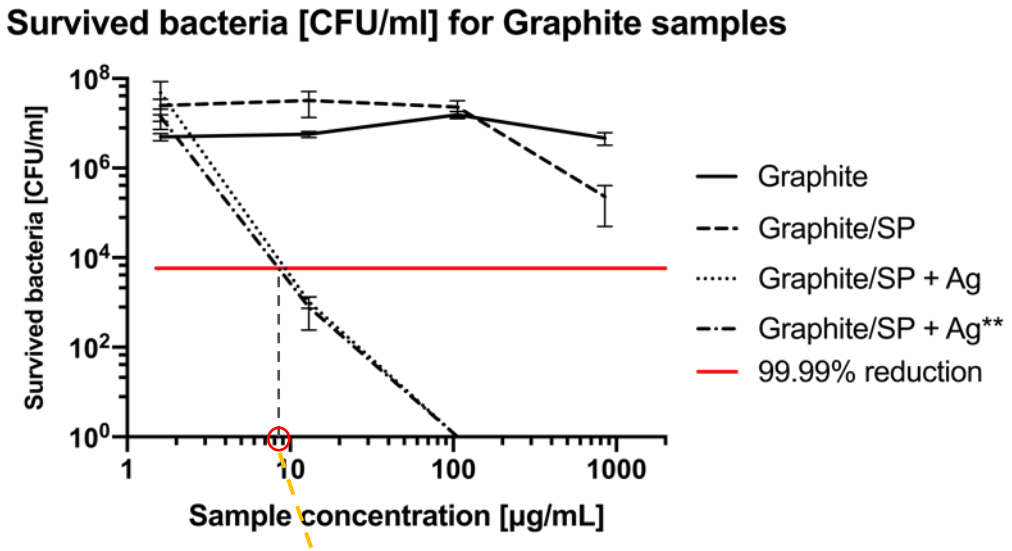


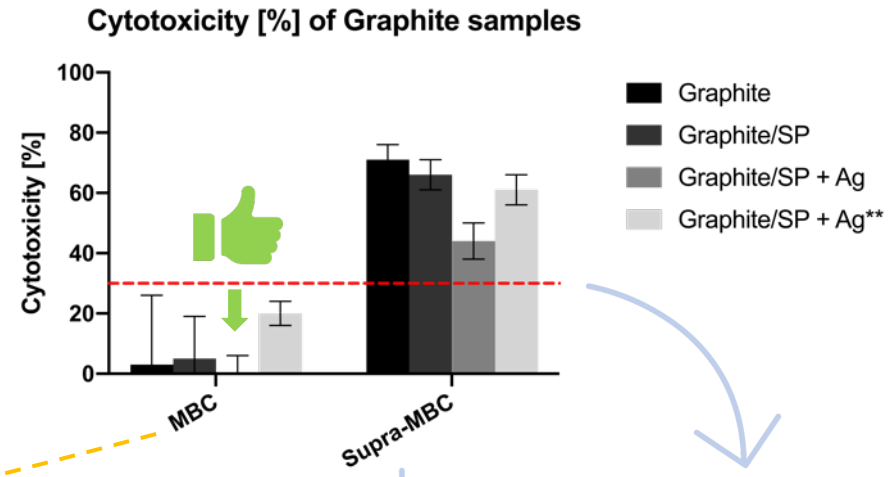


-- : Cytotoxic threshold (30% mortality)

10x MBC

Antimicrobial activity vs. cytotoxicity of materials





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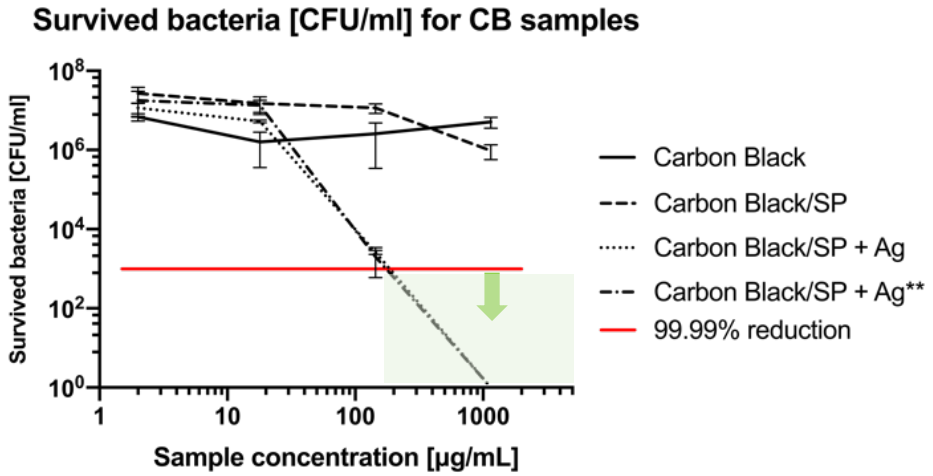
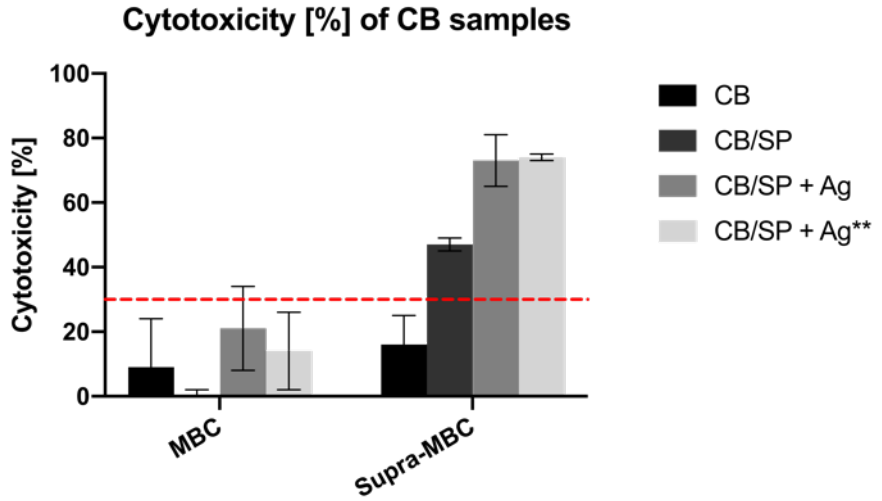
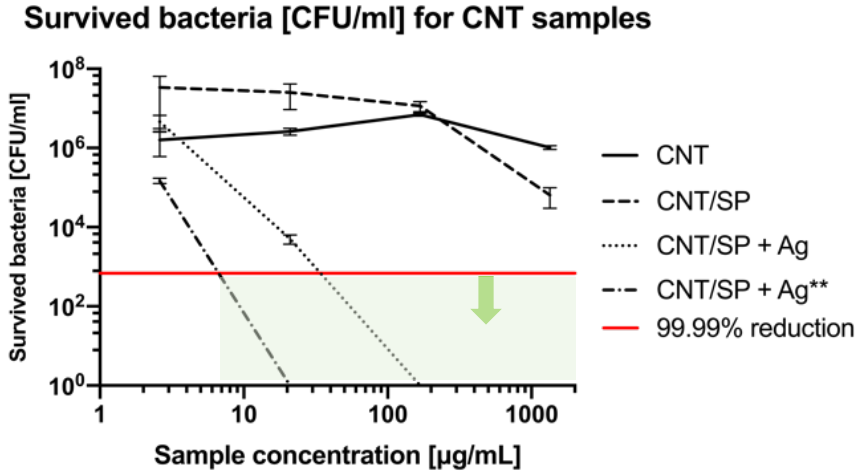
Antimicrobial activity vs. cytotoxicity of materials

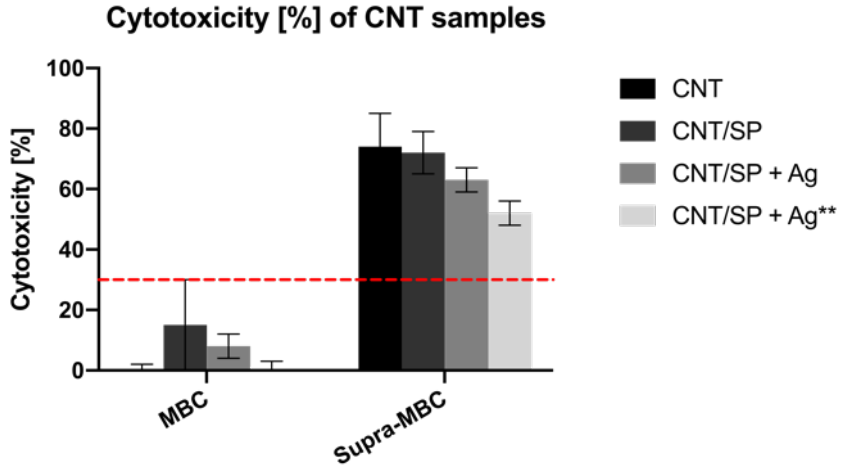
Carbon Black





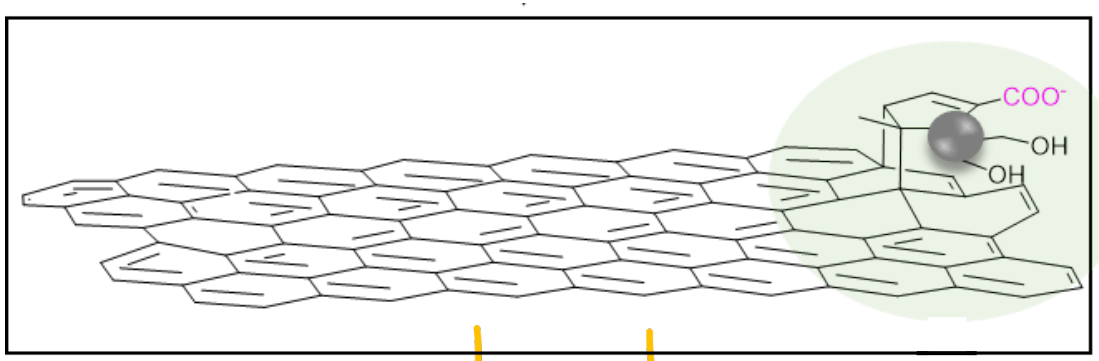
MWCNT





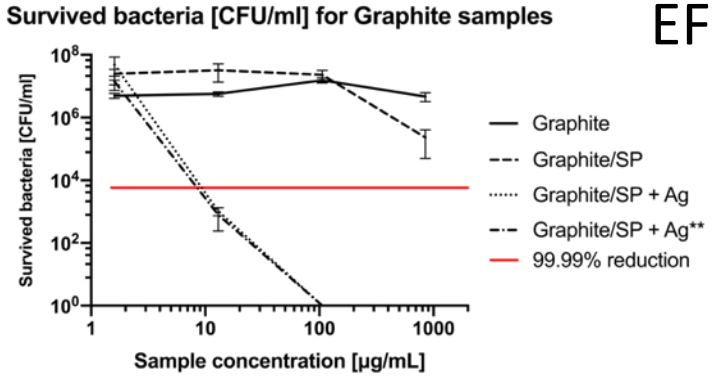
Conclusions

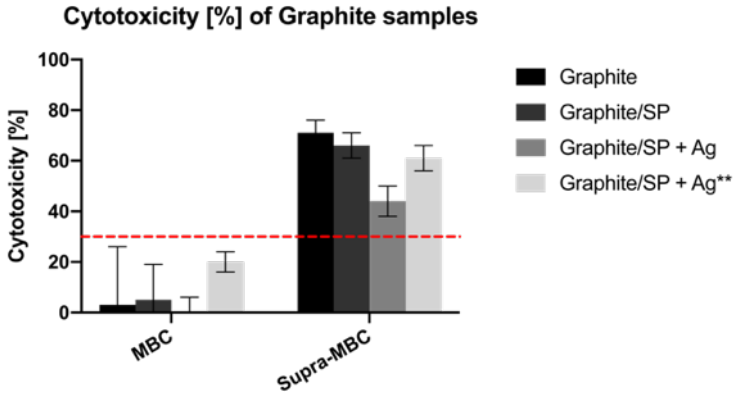
Conclusions



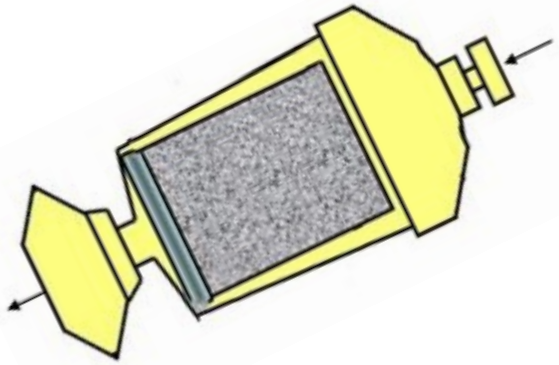
EFFICIENT!

SAFE!

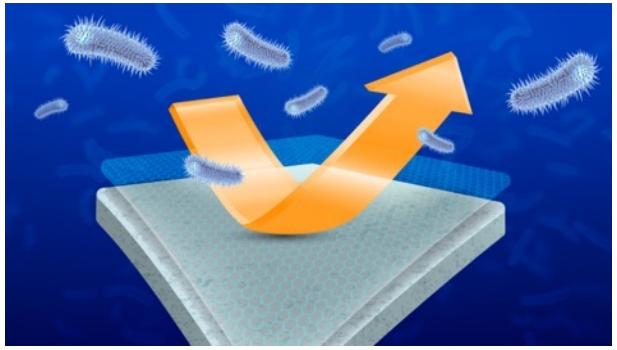




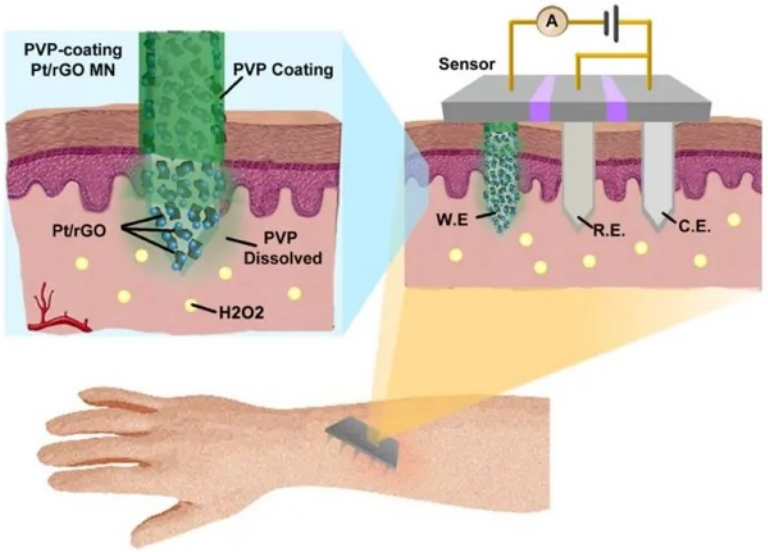
Conclusions



Bactericidal dynamic filters



Antimicrobial coatings



Antimicrobial electronic sensors

Bactericidal blends for tile joints in domestic showers

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Thank you for your kind attention!