ANTIBACTERIAL AND PHOTOTHERMAL PROPERTIES OF SILVER NANOPARTICLES: PAVING THE WAY FOR TARGETED THERAPEUTIC STRATEGIES

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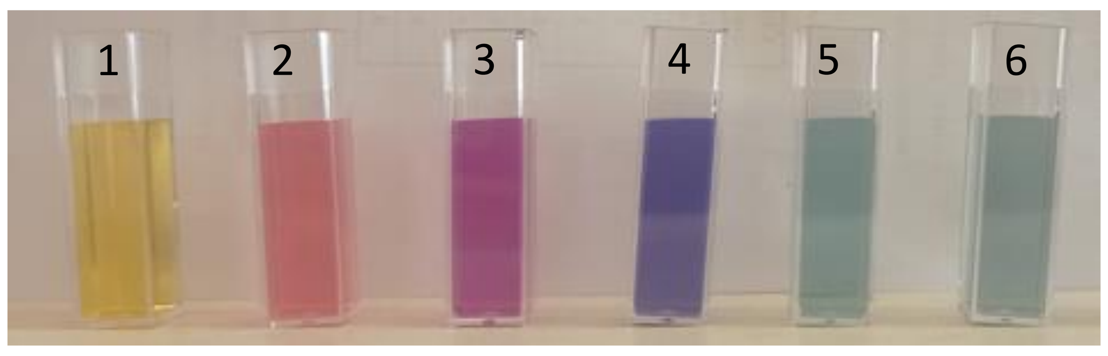
SYNTHESIS OF SILVER NANOPARTICLES

- **Tollens method** $AgNO_3 + NH_3 \rightarrow [Ag(NH_3)_2]^+$
- reduction by various reducing agents:
  - 1 step: maltose (28 nm) vs borohydride (8 nm) AgNPs
  - 2 steps: borohydride & hydrazine + stabilization with sodium citrate
    - precise control of their size, shape, and localized surface plasmon resonance

A



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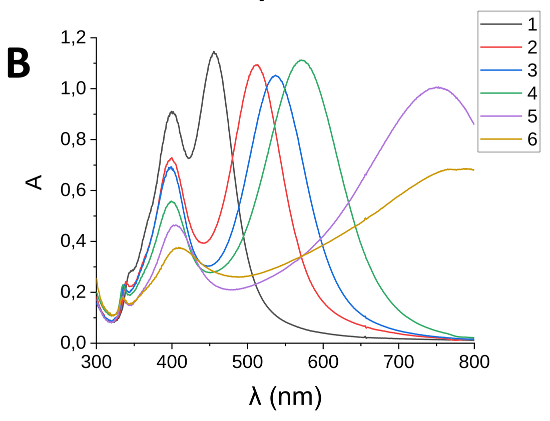


Figure 1. (A) Water dispersion of silver nanoparticle of various sizes and shapes, therefore different optical and plasmonic properties (B).

SILVER DEPOSITION ON CULTIVATION PLATES

Layer-by-layer deposition

- electrostatic interaction between individual layers
- 1. negative layer: 1% poly(acrylic)acid (PAA) – increased hydrophilicity
- 2. positive layer: 1% PDDA - strong electrostatic binding ability of the NPs adsorption of negatively charged nanoparticles

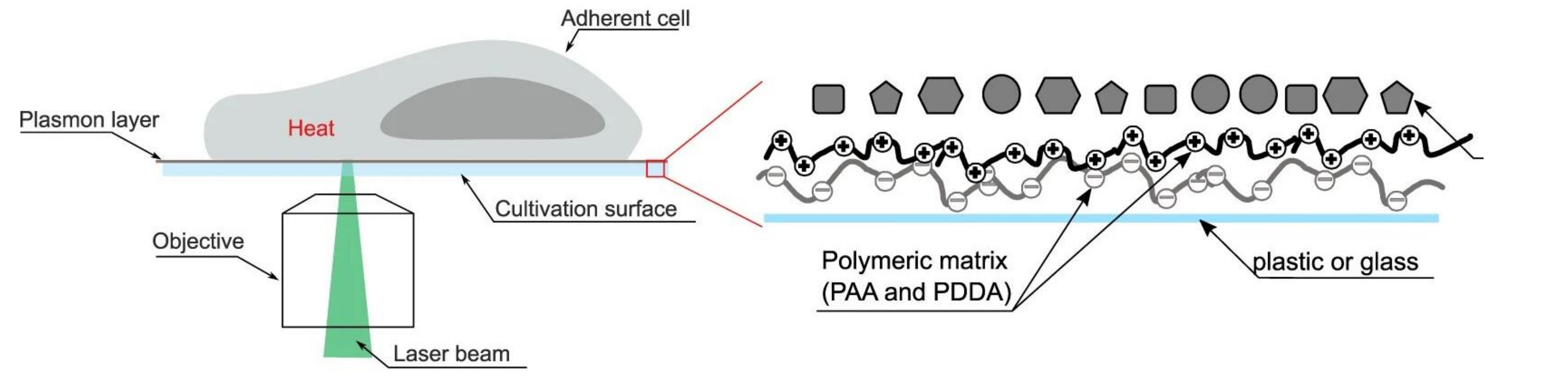


Figure 4. Schematic representation of the concept of microthermal damage inflicted on modified culture plates.

SYNTHESIS OF GCN/Ag NANOCOMPOSITE

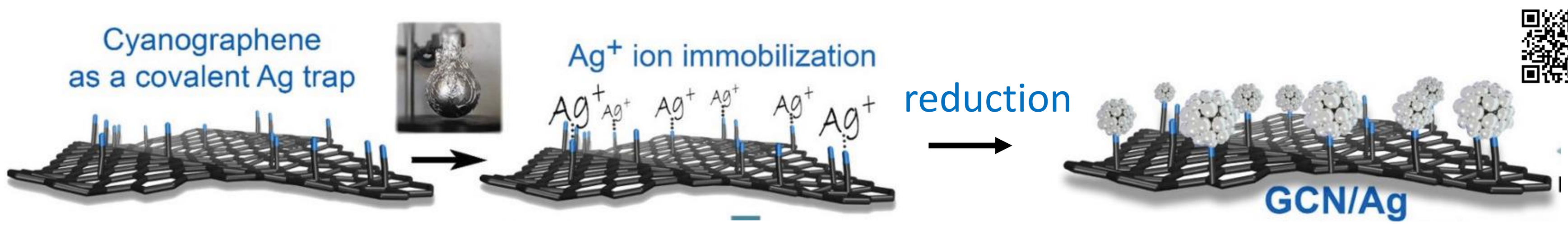
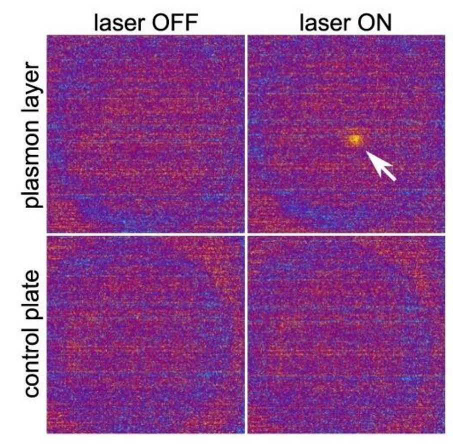


Figure 2. Reaction scheme for the preparation of silver nanoparticles bonded on the nitrile groups of cyanographene (GCN/Ag).

PHOTOTHERMAL PROPERTIES

PROTEIN FOLDING

A



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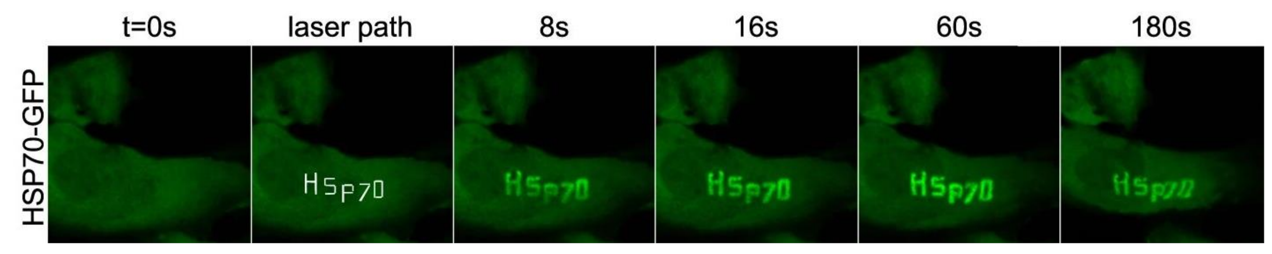
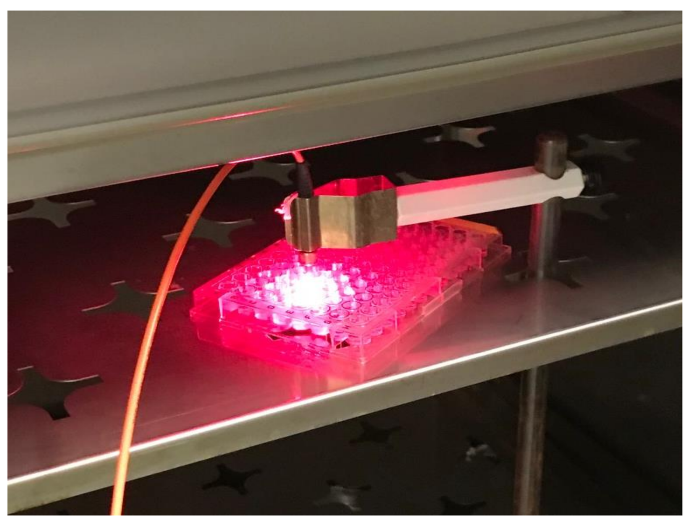


Figure 5A Thermal imaging shows emitted heat detected on non/modified cell plates wells activated by laser (561 nm). **Figure 5B.** Recruitment of various GFP-tagged heat shock related proteins to micro-heated regions. NPs immediately convert energy from light (laser) to heat, which enables direct focusing of the heat on subcellular regions and induces microthermal damage on cellular proteins.

PTT THERAPY

A



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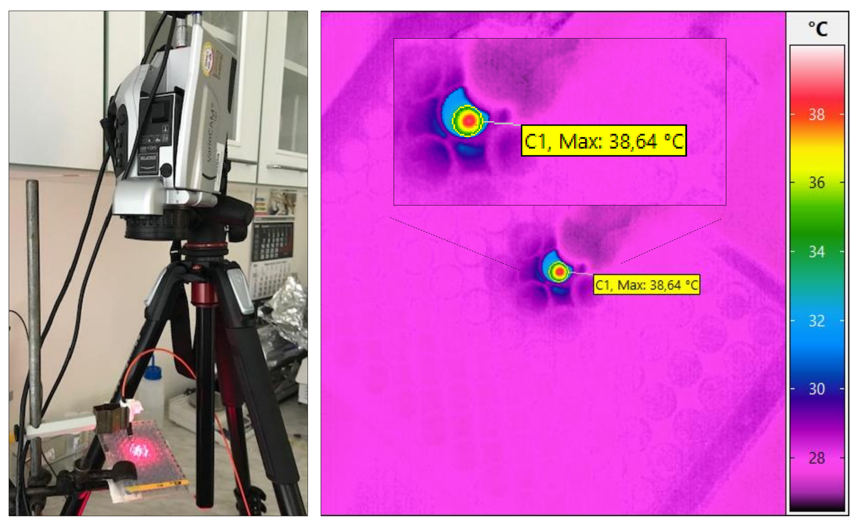
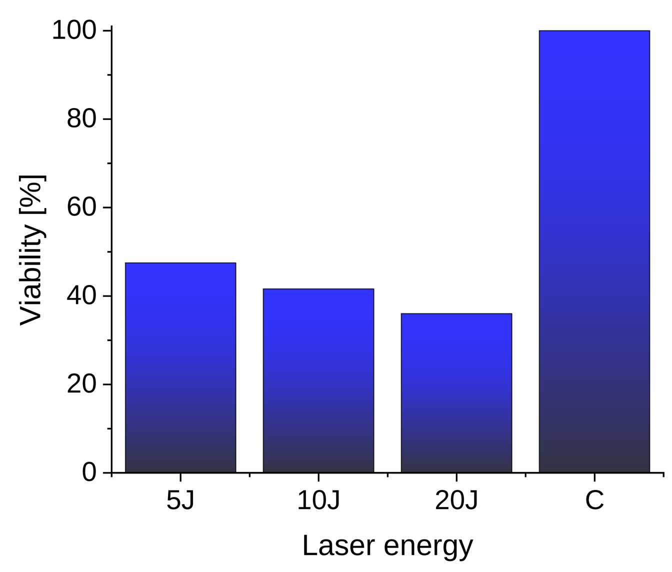


Figure 6A. Irradiation in CO_2 incubator. **Figure 6B.** Experimental setup and temperature increase on Ag-plate after irradiation of the plate, while using 660 nm laser and 20 J irradiation energy.

A



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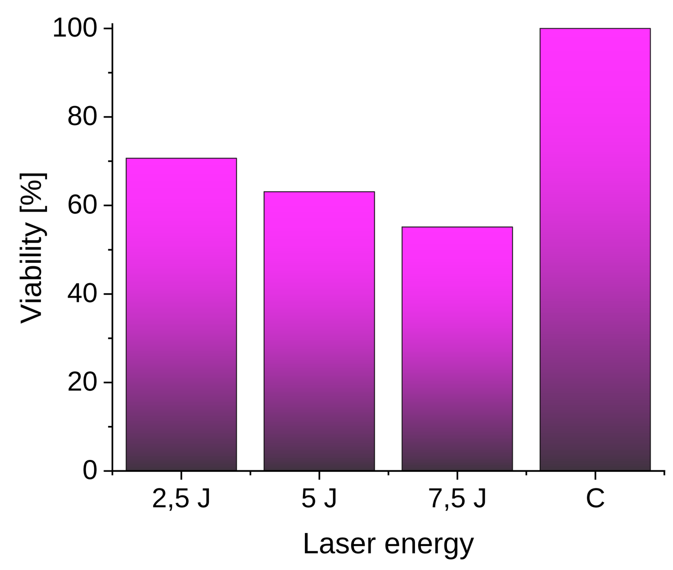

Figure 7. Decreased viability of HeLa cancer cells after irradiation with lasers with different wavelenght (660 nm **(A)**, 730 nm **(B)**) and beam energy in culture plates coated with AgNPs; *C stands for control (cells irradiated with 20 J).

SYNERGISTIC EFFECT

Table 1. Minimum inhibitory concentrations MIC [mg/L] [a] of various antibiotics and the GCN/Ag nanohybrid, average $\overline{FIC}$ values for combinations of antibiotics with the GCN/Ag nanohybrid, and the resulting antibacterial effects.

	E. coli			*P. aeruginosa*			*E. kobei*
	GEN	CTZ	CIP	GEN	CTZ	CIP	COL
ATB alone	128	32	64	8	8	16	64
GCN/Ag alone	1.688	1.688	1.688	1.688	1.688	1.688	3.375
ATB in combination	4-64	1-16	32	0.5-4	1-4	8	1-32
GCN/Ag in combination	0.003 - 0.844	0.211 - 0.844	0.844	0.105 - 0.844	0.844	0.844	0.422 - 1.688
Partial FIC	0.16 - 0.53	0.38 - 0.63	1.00	0.38 - 0.56	0.75 - 1.00	1.00	0.16 - 0.63
$\overline{FIC}$	0.39	0.54	1.00	0.53	0.88	1.00	0.29
Effect	(S)	(PS)	(A)	(PS)	(PS)	(A)	(S)

GEN & GCN/Ag (E. Coli)

	1	2	3	4	5	6	7	8	9	10	11	12	GEN [mg/L]
A	FIC_{AVR}	0,39											256
B	Effect	SYN										MIC GEN	128
C							0,53	0,52	0,51	0,50	0,50		64
D						0,31							32
E					0,25								16
F					0,19								8
G			0,53	0,28	0,16								4
H		MIC GCN/Ag											0
GCN/Ag [mg/L]	3,375	1,688	0,844	0,422	0,211	0,105	0,053	0,026	0,013	0,007	0,003	0	

* (S) synergy, (PS) partial synergy, (A) additive effect

** GCN/Ag shows silver related concentration

Figure 3. Schematic illustrating *E. coli* growth (grey) on microplates in microdilution checkerboard assay. Minimum inhibitory concentration (MIC) for each antimicrobial is determined (blue). FICs are calculated for various combinations antibiotic-GCN/Ag nanohybrid inhibiting bacterial growth and at the same time minimal FIC (green), maximal FIC (red) and average FIC (FIC_{AVR}) are highlighted, and the resulting effect is described (SYN – synergy).

CONCLUSION

- Water dispersion of silver-based NPs of **various size and shape** (i.e., various plasmonic and antibacterial properties) were **synthesized**
- **Culture plates** were **modified** by AgNPs and layer-by-layer method
- Nanoparticles enhanced absorption properties, scattering and conversion of energy to heat, which was directly focused on the individual cells or subcellular compartments (**controllable heating within micro-meter scale**)
- **Microthermal damage of proteins** (tested on U-2-OS cell line expressing a GFO-tagged heat shock protein 70) showed immediate (8 s) microthermal damage following precise laser path pattern, which could be controlled by adjusting the laser power
- **Decreased viability of cancer HeLa cells** were observed after the irradiation with 660 nm and 730 nm laser
- **GCN/Ag nanohybrid** in combination with various antibiotics (e.g., gentamicin, colistin) **enhances antibacterial activity** against resistant strains
- concentration of both antimicrobials is substantionally reduced
- antibiotic becomes effective again

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