

Electronic supplementary information (ESI)

## **Surface properties modulate protein corona formation and determine cellular uptake and cytotoxicity of silver nanoparticles**

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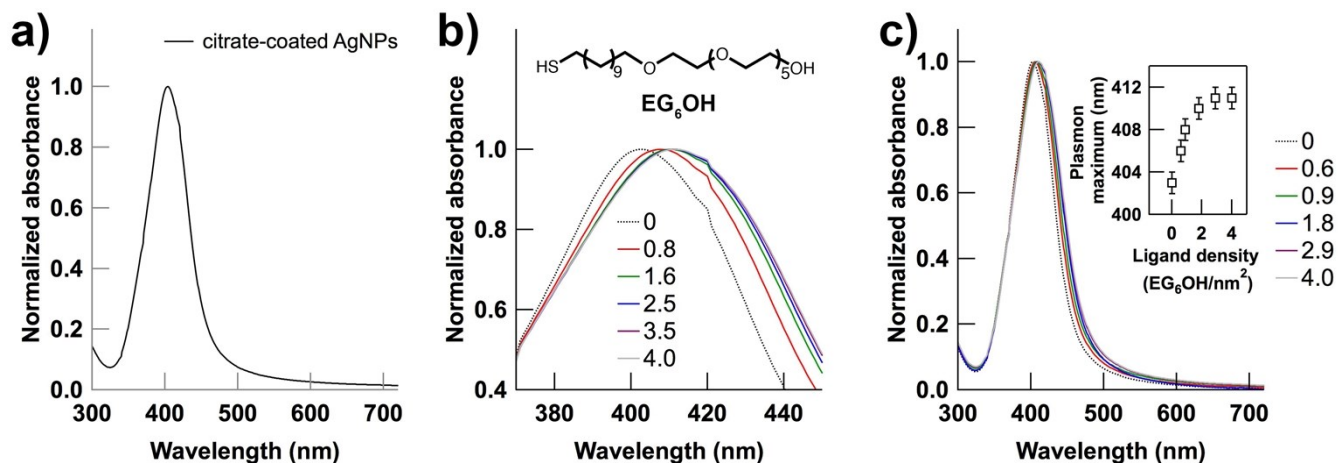
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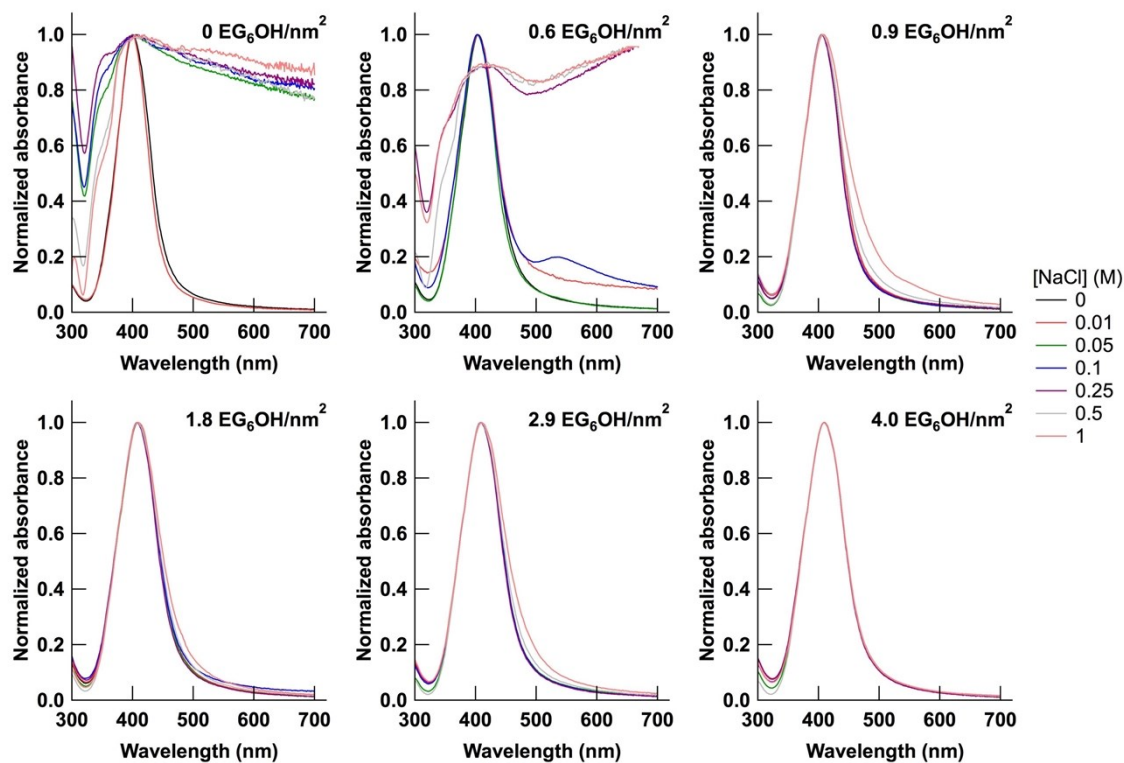
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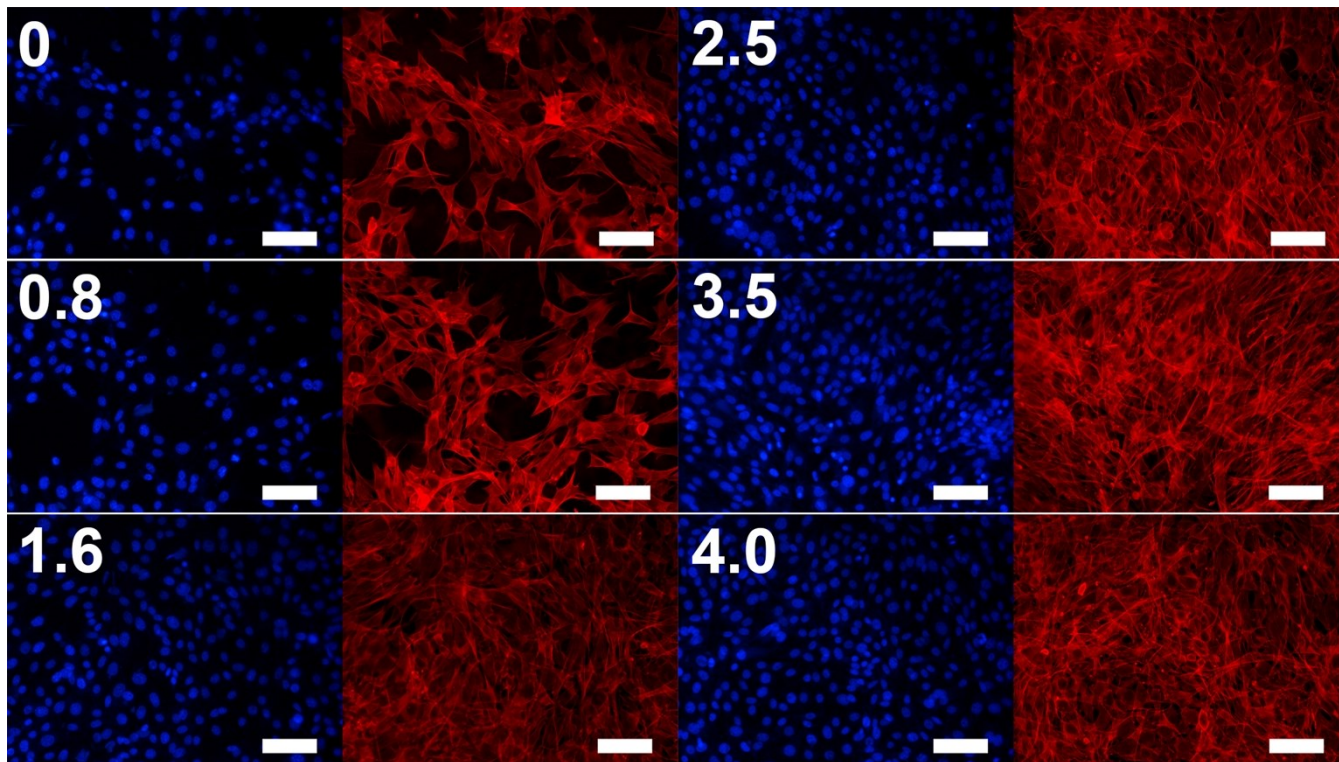
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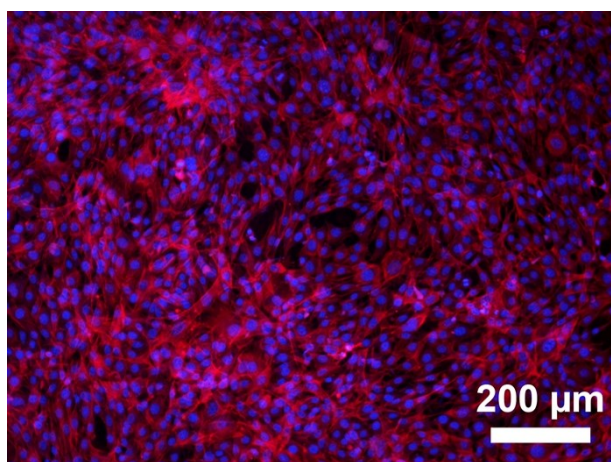
**Fig. S1** (a) UV-Vis absorption spectra of citrate-coated AgNPs in sodium citrate (2 mM). (b) Zoom-in of UV-Vis absorption spectra show in Fig. 1c and molecular structure of EG<sub>6</sub>OH. (c) UV-Vis absorption spectra of AgNPs in sodium citrate (2 mM) as a function of EG<sub>6</sub>OH density of a different batch compared to Fig. 1c (Inset: Dependence of the plasmon band maximum on the EG<sub>6</sub>OH density).



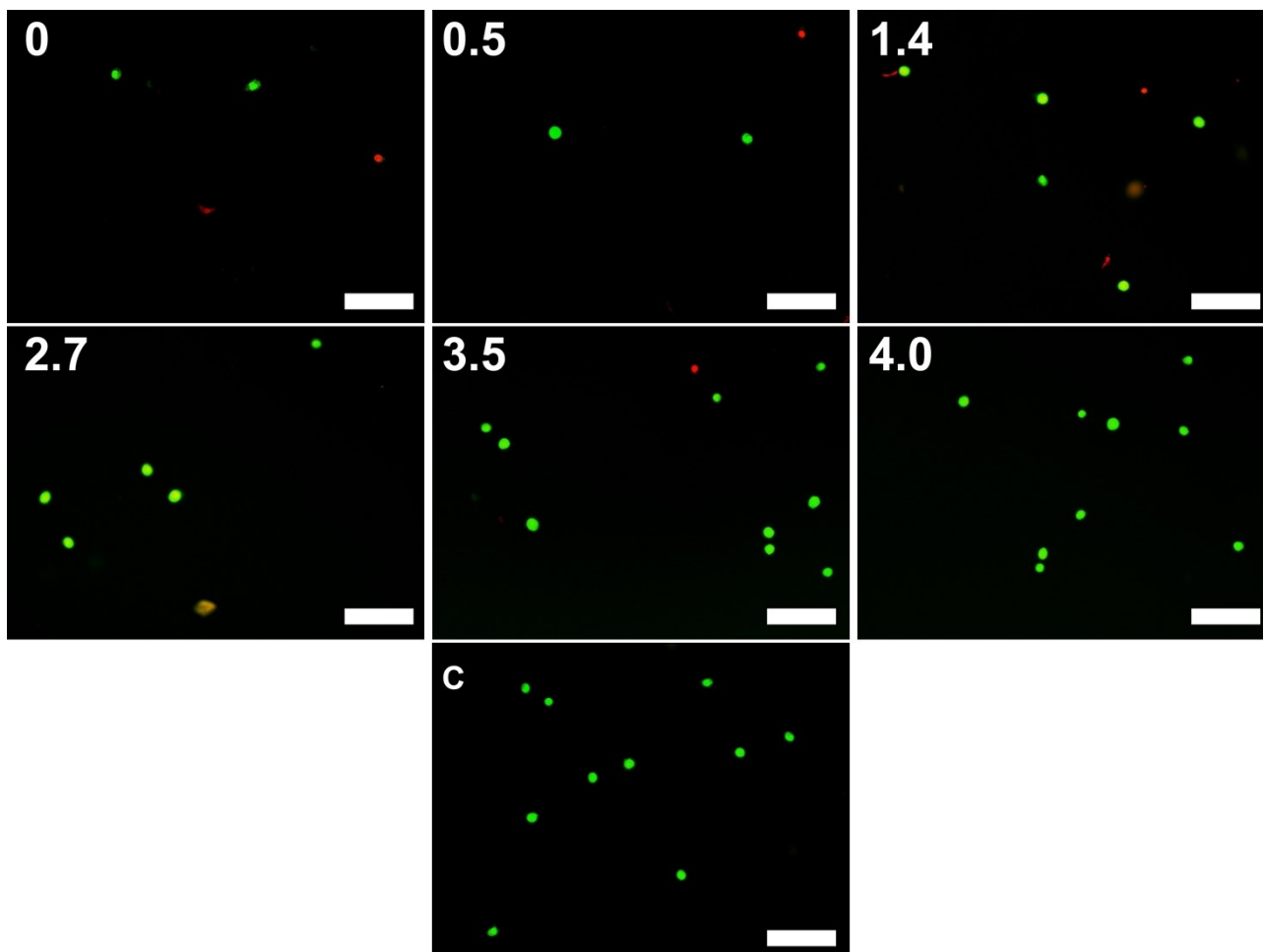
**Fig. S2** UV/Vis absorption spectra of AgNPs with different EG<sub>6</sub>OH grafting density as a function of sodium chloride addition (0-1 M).



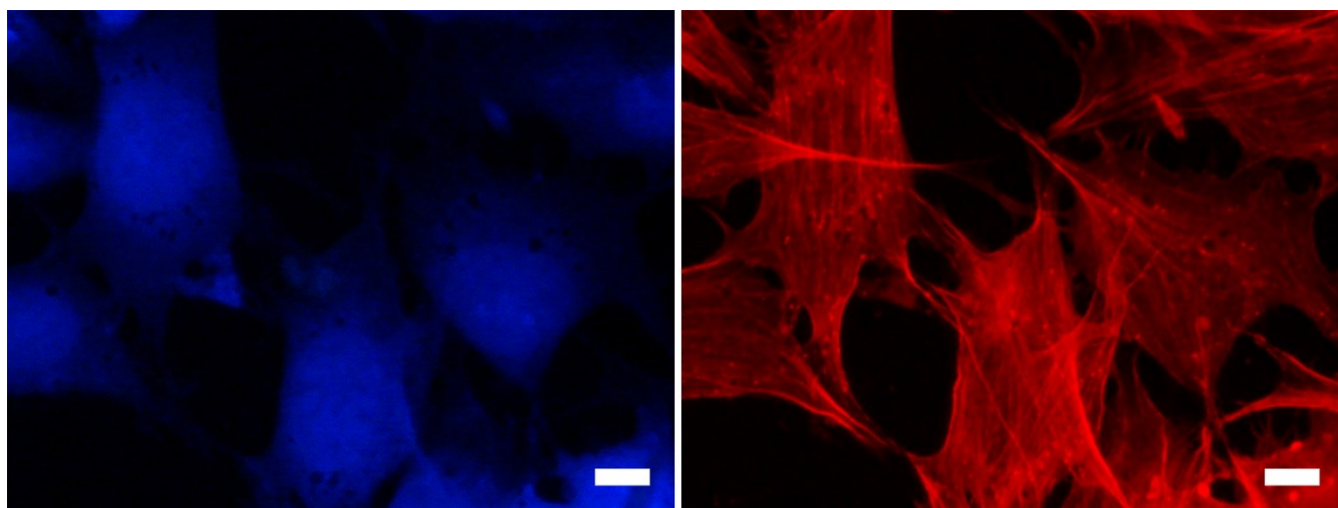
**Fig. S3** Non-merged fluorescence micrographs of cells labeled specifically for actin (red, right) and the nucleus (blue, left) after 24 h of incubation with 40  $\mu\text{g/mL}$  of AgNPs as a function ligand density ( $\text{EG}_6\text{OH}/\text{nm}^2$ ) (Scale bar: 100  $\mu\text{m}$ ).



**Fig. S4** Fluorescence micrographs of NIH-3T3 cells labeled specifically for actin (red) and the nucleus (blue) after 24h of incubation with vehicle solution (control).



**Fig. S5** Morphological observation of NIH-3T3 cells treated with 40  $\mu\text{g}/\text{mL}$  of AgNPs or vehicle solution (c) for 48h after AO/EB staining as a function ligand density ( $\text{EG}_6\text{OH}/\text{nm}^2$ ).



**Fig. S6** Non-merged fluorescence micrographs of cells labeled specifically for actin (red, right) and the nucleus (blue, left) after 24 h of incubation with 40  $\mu\text{g}/\text{mL}$  of AgNPs with ligand density 0  $\text{EG}_6\text{OH}/\text{nm}^2$  (Scale bar: 10  $\mu\text{m}$ ).

**Table S1:** Physicochemical properties of nanoparticles before and after serum exposure

Ligand density (EG <sub>6</sub> OH/nm <sup>2</sup> )	Hydrodynamic diameter (nm)		Zeta potential (mV)	
	Before serum exposure	After serum exposure	Before serum exposure	After serum exposure
0	26±1	88±5	-40±2	-29±1
0.7	28±1	50±1	-37±2	-29±1
1.5	27±1	37±1	-34±2	-30±1
2.2	29±1	33±2	-32±2	-30±2
3.0	28±1	34±1	-32±1	-28±1
3.7	30±1	34±1	-29±1	-29±3
4.0	30±1	32±1	-29±1	-30±2

Data are presented as mean ± standard deviation (SD)

**Table S2:** List of all proteins identified by LC-MS/MS in the corona of citrate-coated AgNPs following exposure to the cell growth media.

Abbreviation	Description	MW (kDa)	emPAI	% mol
ALBU_BOVIN	Serum albumin	71.244	2.3	21.5
APOE_BOVIN	Apolipoprotein E	36.015	1.76	16.4
HBA_BOVIN	Hemoglobin subunit alpha	15.175	1.11	10.4
ANT3_BOVIN	Antithrombin-III	52.827	0.62	5.8
FETUA_BOVIN	Alpha-2-HS-glycoprotein	39.193	0.61	5.7
TSP1_BOVIN	Thrombospondin-1	133.442	0.43	4.0
HBBF_BOVIN	Hemoglobin fetal subunit beta	15.963	0.41	3.8
GELS_BOVIN	Gelsolin	80.966	0.39	3.6
C1QB_BOVIN	Complement C1q subcomponent subunit B	26.611	0.37	3.5
APOA1_BOVIN	Apolipoprotein A-I	30.258	0.32	3.0
K1C17_BOVIN	Keratin, type I cytoskeletal 17	48.967	0.31	2.9
A1AT_BOVIN	Alpha-1-antitrypsin	46.417	0.26	2.4
DDBX_BOVIN	Dihydrodiol dehydrogenase	37.046	0.26	2.4
THRB_BOVIN	Prothrombin	71.886	0.25	2.3
PLMN_BOVIN	Plasminogen	93.894	0.13	1.2
F13A_BOVIN	Coagulation factor XIII A chain (Fragment)	22.844	0.13	1.2
CO3_BOVIN	Complement C3	188.675	0.11	1.0
C1QA_BOVIN	Complement C1q subcomponent subunit A	26.014	0.11	1.0
GSTA2_BOVIN	Glutathione S-transferase A2	25.814	0.11	1.0
CO4_BOVIN	Complement C4 (Fragments)	103.018	0.09	0.8
K2C5_BOVIN	Keratin, type II cytoskeletal 5	63.069	0.09	0.8
RGN_BOVIN	Regucalcin	33.857	0.09	0.8
FA5_BOVIN	Coagulation factor V	249.912	0.08	0.7
HSP7C_BOVIN	Heat shock cognate 71 kDa protein	71.424	0.08	0.7
LDHA_BOVIN	L-lactate dehydrogenase A chain	36.916	0.08	0.7
ALDOB_BOVIN	Fructose-bisphosphate aldolase B	39.917	0.07	0.7
CBPB2_BOVIN	Carboxypeptidase B2	49.247	0.06	0.6
HS71A_BOVIN	Heat shock 70 kDa protein 1A	70.259	0.04	0.4
CO7_BOVIN	Complement component C7	96.223	0.03	0.3



**Table S3:** List of all proteins identified by LC-MS/MS in bands 2, 3, 4, 6, and 7 in Figure 2c of the corona of citrate-coated AgNPs following exposure to the cell growth media.

Abbreviation	Description	MW (kDa)	emPAI	% mol
APOE_BOVIN	Apolipoprotein E	36.015	1.76	19.8
ALBU_BOVIN	Serum albumin	71.244	1.35	15.2
HBA_BOVIN	Hemoglobin subunit alpha	15.175	1.11	12.5
ANT3_BOVIN	Antithrombin-III	52.827	0.62	7.0
TSP1_BOVIN	Thrombospondin-1	133.442	0.43	4.8
HBBF_BOVIN	Hemoglobin fetal subunit beta	15.963	0.41	4.6
GELS_BOVIN	Gelsolin	80.966	0.39	4.4
C1QB_BOVIN	Complement C1q subcomponent subunit B	26.611	0.37	4.2
APOA1_BOVIN	Apolipoprotein A-I	30.258	0.32	3.6
K1C17_BOVIN	Keratin, type I cytoskeletal 17	48.967	0.31	3.5
DDBX_BOVIN	Dihydrodiol dehydrogenase	37.046	0.26	2.9
THRB_BOVIN	Prothrombin	71.886	0.25	2.8
PLMN_BOVIN	Plasminogen	93.894	0.13	1.5
F13A_BOVIN	Coagulation factor XIII A chain (Fragment)	22.844	0.13	1.5
C1QA_BOVIN	Complement C1q subcomponent subunit A	26.014	0.11	1.2
GSTA2_BOVIN	Glutathione S-transferase A2	25.814	0.11	1.2
CO4_BOVIN	Complement C4 (Fragments)	103.018	0.09	1.0
K2C5_BOVIN	Keratin, type II cytoskeletal 5	63.069	0.09	1.0
RGN_BOVIN	Regucalcin	33.857	0.09	1.0
FA5_BOVIN	Coagulation factor V	249.912	0.08	0.9
HSP7C_BOVIN	Heat shock cognate 71 kDa protein	71.424	0.08	0.9
LDHA_BOVIN	L-lactate dehydrogenase A chain	36.916	0.08	0.9
ALDOB_BOVIN	Fructose-bisphosphate aldolase B	39.917	0.07	0.8
FETUA_BOVIN	Alpha-2-HS-glycoprotein	39.193	0.07	0.8
CBPB2_BOVIN	Carboxypeptidase B2	49.247	0.06	0.7
A1AT_BOVIN	Alpha-1-antitrypsin	46.417	0.06	0.7
HS71A_BOVIN	Heat shock 70 kDa protein 1A	70.5	0.04	0.4
CO7_BOVIN	Complement component C7	96.223	0.03	0.3

**Table S4:** List of all proteins identified by LC-MS/MS in band 6 in Figure 2c of the corona of citrate-coated AgNPs following exposure to the cell growth media.

Abbreviation	Description	MW (kDa)	emPAI	% mol
APOE_BOVIN	Apolipoprotein E	36.015	1.76	58.5
DDBX_BOVIN	Dihydrodiol dehydrogenase	37.046	0.26	8.6
HBA_BOVIN	Hemoglobin subunit alpha	15.175	0.20	6.6
K1C17_BOVIN	Keratin, type I cytoskeletal 17	48.967	0.19	6.3
THRB_BOVIN	Prothrombin	71.886	0.17	5.6
RGN_BOVIN	Regucalcin	33.857	0.09	3.0
ALBU_BOVIN	Serum albumin	71.244	0.08	2.7
LDHA_BOVIN	L-lactate dehydrogenase A chain	36.916	0.08	2.7
ALDOB_BOVIN	Fructose-bisphosphate aldolase B	39.917	0.07	2.3
CBPB2_BOVIN	Carboxypeptidase B2	49.247	0.06	2.0
GELS_BOVIN	Gelsolin	80.966	0.04	1.3
FA5_BOVIN	Coagulation factor V	249.912	0.01	0.3

**Table S5:** NIH-3T3 nuclei per unit area after 24 h of incubation with 40  $\mu\text{g/mL}$  of AgNPs as a function ligand density ( $\text{EG}_6\text{OH}/\text{nm}^2$ ).

Ligand density ( $\text{EG}_6\text{OH}/\text{nm}^2$ )	Cell nuclei density (n.ro/ $\text{mm}^2$ )
0	493 $\pm$ 53
0.8	705 $\pm$ 86
1.6	994 $\pm$ 105
2.5	1084 $\pm$ 174
3.5	1280 $\pm$ 102
4.0	1485 $\pm$ 187
Control (vehicle solution)	1537 $\pm$ 50

Data are presented as mean  $\pm$  standard deviation (SD) of at least three independent culture experiments.



**Table S6:** Percentage of viable cells, early apoptosis, late apoptosis and necrotic cells after 48 h of incubation with 40  $\mu\text{g/mL}$  of AgNPs as a function ligand density ( $\text{EG}_6\text{OH}/\text{nm}^2$ ).

Ligand density ( $\text{EG}_6\text{OH}/\text{nm}^2$ )	Live cells	Early apoptosis	Late apoptosis	Necrosis
0	15%	67%	7%	11%
0.5	20%	62%	5%	13%
1.4	30%	52%	3%	15%
2.7	54%	35%	2%	9%
3.5	77%	16%	2%	5%
4.0	85%	12%	1%	2%
Control (vehicle solution)	96%	2%	0%	2%

Data are presented as mean of at least three independent culture experiments.