Supplementary Materials

Nanoparticles influence lytic phage T4-like performance *in vitro*

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X-ray diffraction patterns of synthesized SiO_2-Fe_3O_4-TiO_2 particles

The X-ray diffraction patterns of synthesised SiO_2-Fe_3O_4-TiO_2 particles are presented in the Figure S1. From the presented pasterns characteristic peaks were assigned to the magnetite phase of iron oxide (JCPDS no. 65-3107), anatase (ICDD no. 03-065-5714) and rutile (ICDD no. 03-065-1118) phase of titanium dioxide. According to the TEM analysis, particles core is made from iron oxide and titanium dioxide is placed on the thin layer of mesoporous silica and small agglomerates form on the surface of the silica shell. Due to this, the prevailing intensity of the iron oxide over titanium dioxide peaks is in agreement with the TEM analysis of the SiO_2-Fe_3O_4-TiO_2 particles.

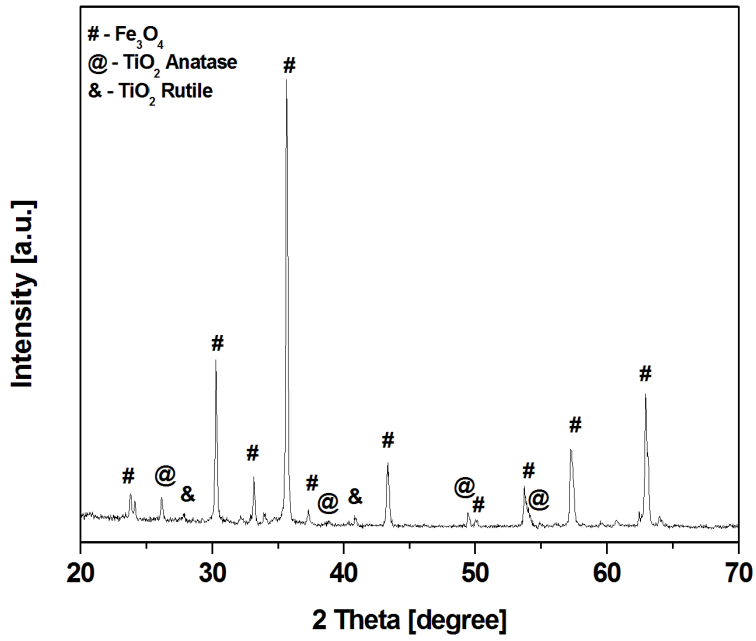

Figure S1. X-ray diffraction spectra of SiO_2-Fe_3O_4-TiO_2 particles with marked peaks corresponding to the Fe_3O_4 and TiO_2 phases.

Exemplary Petri plate from the coincubation test

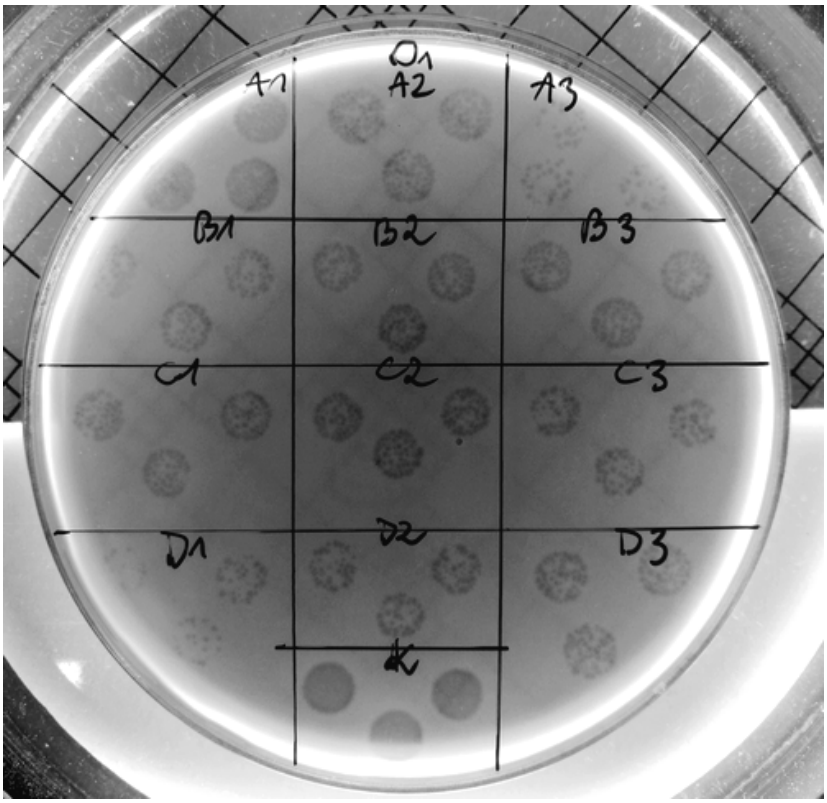

Figure S2. Detailed visualization of the results plate from the coincubation test.

E. coli lysis curves of the tested phage and nanoparticles

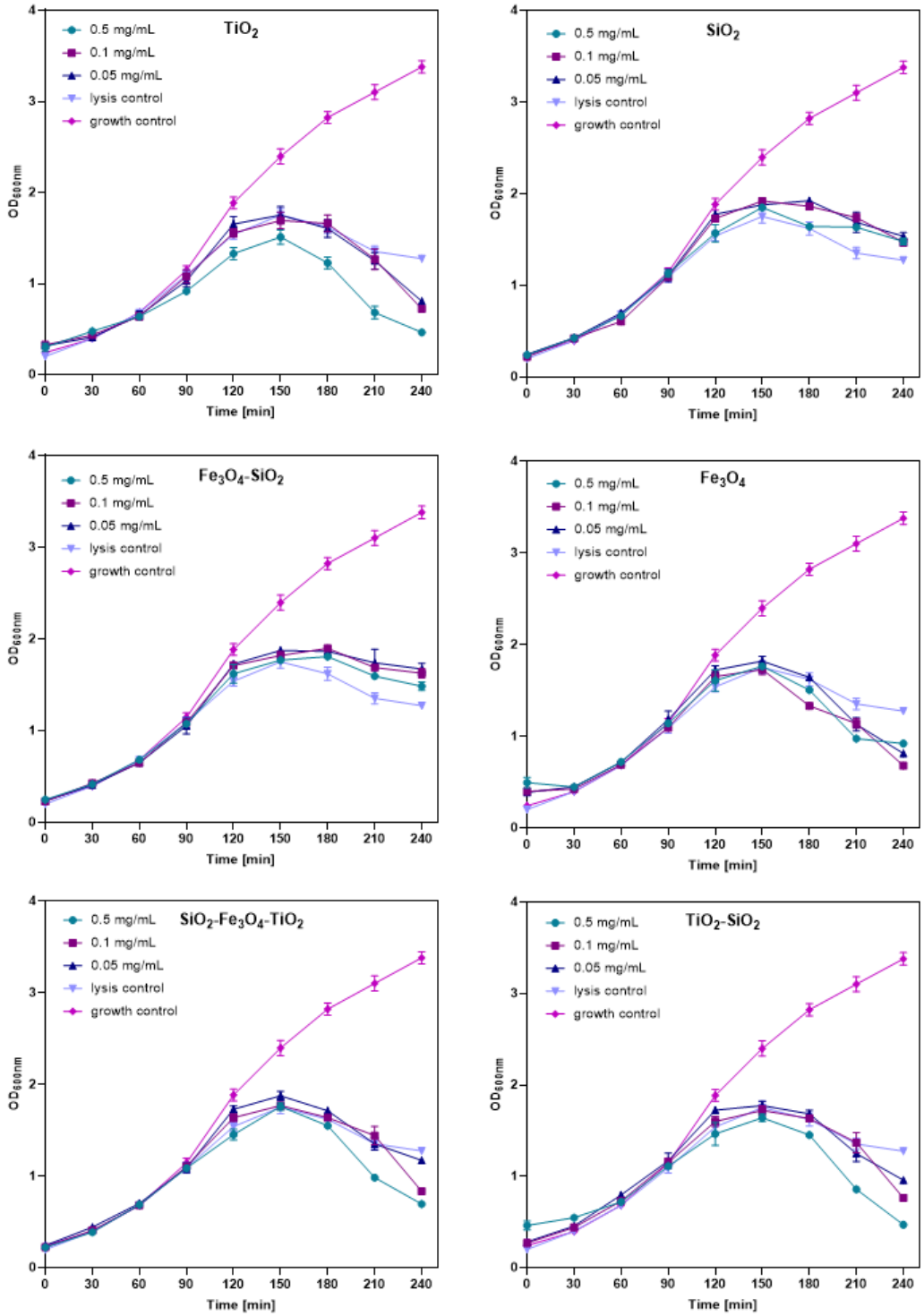

Figure S3. Lysis profile experiments of phage T4$_5$ infection in the presence of nanoparticles at different concentrations (0.5, 0.1 and 0.05 mg/mL).

Detailed statistical results of the phage eclipse periods test

Table S1. Dunnett's multiple comparisons test for significance interpretation of the results of progeny phage numbers, from the test of phage eclipse periods after nanoparticles exposure.

Dunnett's multiple comparisons test	Significant?	Summary	Adjusted P Value
0.5 mg/mL			
0 min			
control vs. TiO_2	No	ns	0,8610
control vs. Fe_3O_4-SiO_2	No	ns	0,9998
control vs. SiO_2	No	ns	0,9995
control vs. Fe_3O_4	No	ns	0,9996
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9977
control vs. TiO_2-SiO_2	No	ns	0,9975
2 min			
control vs. TiO_2	No	ns	0,9997
control vs. Fe_3O_4-SiO_2	No	ns	>0,9999
control vs. SiO_2	No	ns	0,8062
control vs. Fe_3O_4	No	ns	0,9975
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	>0,9999
control vs. TiO_2-SiO_2	No	ns	0,9997
4 min			
control vs. TiO_2	No	ns	0,9996
control vs. Fe_3O_4-SiO_2	No	ns	0,9996
control vs. SiO_2	No	ns	0,8279
control vs. Fe_3O_4	No	ns	0,9998
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	>0,9999
control vs. TiO_2-SiO_2	No	ns	>0,9999
6 min			
control vs. TiO_2	No	ns	>0,9999
control vs. Fe_3O_4-SiO_2	No	ns	0,9975
control vs. SiO_2	Yes	**	0,0021
control vs. Fe_3O_4	No	ns	0,9461
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9959
control vs. TiO_2-SiO_2	No	ns	0,9591
8 min			
control vs. TiO_2	No	ns	0,9619
control vs. Fe_3O_4-SiO_2	No	ns	0,9959
control vs. SiO_2	No	ns	0,9309
control vs. Fe_3O_4	No	ns	0,9925
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,8646
control vs. TiO_2-SiO_2	No	ns	0,8646
10 min			
control vs. TiO_2	No	ns	0,7215
control vs. Fe_3O_4-SiO_2	No	ns	0,3828

control vs. SiO_2	No	ns	0,9724
control vs. Fe_3O_4	No	ns	0,9933
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9882
control vs. TiO_2-SiO_2	No	ns	0,2317
12 min			
control vs. TiO_2	Yes	**	0,0040
control vs. Fe_3O_4-SiO_2	Yes	****	<0,0001
control vs. SiO_2	Yes	*	0,0157
control vs. Fe_3O_4	Yes	**	0,0018
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
14 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	Yes	****	<0,0001
control vs. SiO_2	Yes	****	<0,0001
control vs. Fe_3O_4	Yes	****	<0,0001
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
16 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	Yes	****	<0,0001
control vs. SiO_2	Yes	****	<0,0001
control vs. Fe_3O_4	Yes	****	<0,0001
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
0.1 mg/mL			
0 min			
control vs. TiO_2	No	ns	0,9998
control vs. Fe_3O_4-SiO_2	No	ns	0,9919
control vs. SiO_2	No	ns	0,9996
control vs. Fe_3O_4	No	ns	0,9996
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9997
control vs. TiO_2-SiO_2	No	ns	0,7349
2 min			
control vs. TiO_2	No	ns	0,6667
control vs. Fe_3O_4-SiO_2	No	ns	0,4407
control vs. SiO_2	No	ns	0,9682
control vs. Fe_3O_4	No	ns	0,9996
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,8565
control vs. TiO_2-SiO_2	No	ns	>0,9999
4 min			
control vs. TiO_2	No	ns	0,9977
control vs. Fe_3O_4-SiO_2	No	ns	0,3916
control vs. SiO_2	No	ns	0,4533
control vs. Fe_3O_4	No	ns	0,9998
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,8112
control vs. TiO_2-SiO_2	No	ns	0,9958
6 min			
control vs. TiO_2	No	ns	0,8910
control vs. Fe_3O_4-SiO_2	No	ns	0,3627

control vs. SiO_2	No	ns	0,6458
control vs. Fe_3O_4	No	ns	0,9206
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,5078
control vs. TiO_2-SiO_2	No	ns	0,9998
8 min			
control vs. TiO_2	No	ns	0,4158
control vs. Fe_3O_4-SiO_2	Yes	*	0,0260
control vs. SiO_2	No	ns	0,4158
control vs. Fe_3O_4	No	ns	0,9059
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,1263
control vs. TiO_2-SiO_2	No	ns	0,8530
10 min			
control vs. TiO_2	No	ns	0,2635
control vs. Fe_3O_4-SiO_2	No	ns	0,3123
control vs. SiO_2	No	ns	0,7395
control vs. Fe_3O_4	No	ns	0,9469
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,2635
control vs. TiO_2-SiO_2	No	ns	0,7148
12 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	No	ns	0,0875
control vs. SiO_2	Yes	***	0,0002
control vs. Fe_3O_4	Yes	**	0,0020
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
14 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	No	ns	0,9581
control vs. SiO_2	Yes	****	<0,0001
control vs. Fe_3O_4	Yes	****	<0,0001
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
16 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	Yes	**	0,0016
control vs. SiO_2	Yes	****	<0,0001
control vs. Fe_3O_4	Yes	****	<0,0001
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
0.05 mg/mL			
0 min			
control vs. TiO_2	No	ns	0,9998
control vs. Fe_3O_4-SiO_2	No	ns	0,7528
control vs. SiO_2	No	ns	0,9935
control vs. Fe_3O_4	No	ns	0,9917
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9969
control vs. TiO_2-SiO_2	No	ns	0,8973
2 min			
control vs. TiO_2	No	ns	0,4734
control vs. Fe_3O_4-SiO_2	No	ns	0,9998

control vs. SiO_2	No	ns	0,5806
control vs. Fe_3O_4	No	ns	0,9807
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9935
control vs. TiO_2-SiO_2	No	ns	0,9994
4 min			
control vs. TiO_2	No	ns	0,9997
control vs. Fe_3O_4-SiO_2	Yes	****	<0,0001
control vs. SiO_2	No	ns	0,9976
control vs. Fe_3O_4	No	ns	0,9877
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9667
control vs. TiO_2-SiO_2	No	ns	0,9788
6 min			
control vs. TiO_2	No	ns	0,8987
control vs. Fe_3O_4-SiO_2	No	ns	0,9797
control vs. SiO_2	No	ns	0,9976
control vs. Fe_3O_4	No	ns	0,8434
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,9246
control vs. TiO_2-SiO_2	No	ns	0,9976
8 min			
control vs. TiO_2	No	ns	0,8796
control vs. Fe_3O_4-SiO_2	No	ns	0,7104
control vs. SiO_2	No	ns	0,8796
control vs. Fe_3O_4	No	ns	0,9979
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,0762
control vs. TiO_2-SiO_2	No	ns	0,8796
10 min			
control vs. TiO_2	No	ns	0,3116
control vs. Fe_3O_4-SiO_2	Yes	*	0,0133
control vs. SiO_2	No	ns	0,5007
control vs. Fe_3O_4	No	ns	0,9870
control vs. SiO_2-Fe_3O_4-TiO_2	No	ns	0,0588
control vs. TiO_2-SiO_2	No	ns	0,3631
12 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	Yes	****	<0,0001
control vs. SiO_2	Yes	****	<0,0001
control vs. Fe_3O_4	Yes	**	0,0045
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
14 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	Yes	****	<0,0001
control vs. SiO_2	Yes	****	<0,0001
control vs. Fe_3O_4	Yes	****	<0,0001
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001
16 min			
control vs. TiO_2	Yes	****	<0,0001
control vs. Fe_3O_4-SiO_2	Yes	****	<0,0001
control vs. SiO_2	Yes	****	<0,0001

control vs. Fe_3O_4	Yes	****	<0,0001
control vs. SiO_2-Fe_3O_4-TiO_2	Yes	****	<0,0001
control vs. TiO_2-SiO_2	Yes	****	<0,0001

ns – not significant

Additional TEM images of the phage and nanoparticles physical attachments.

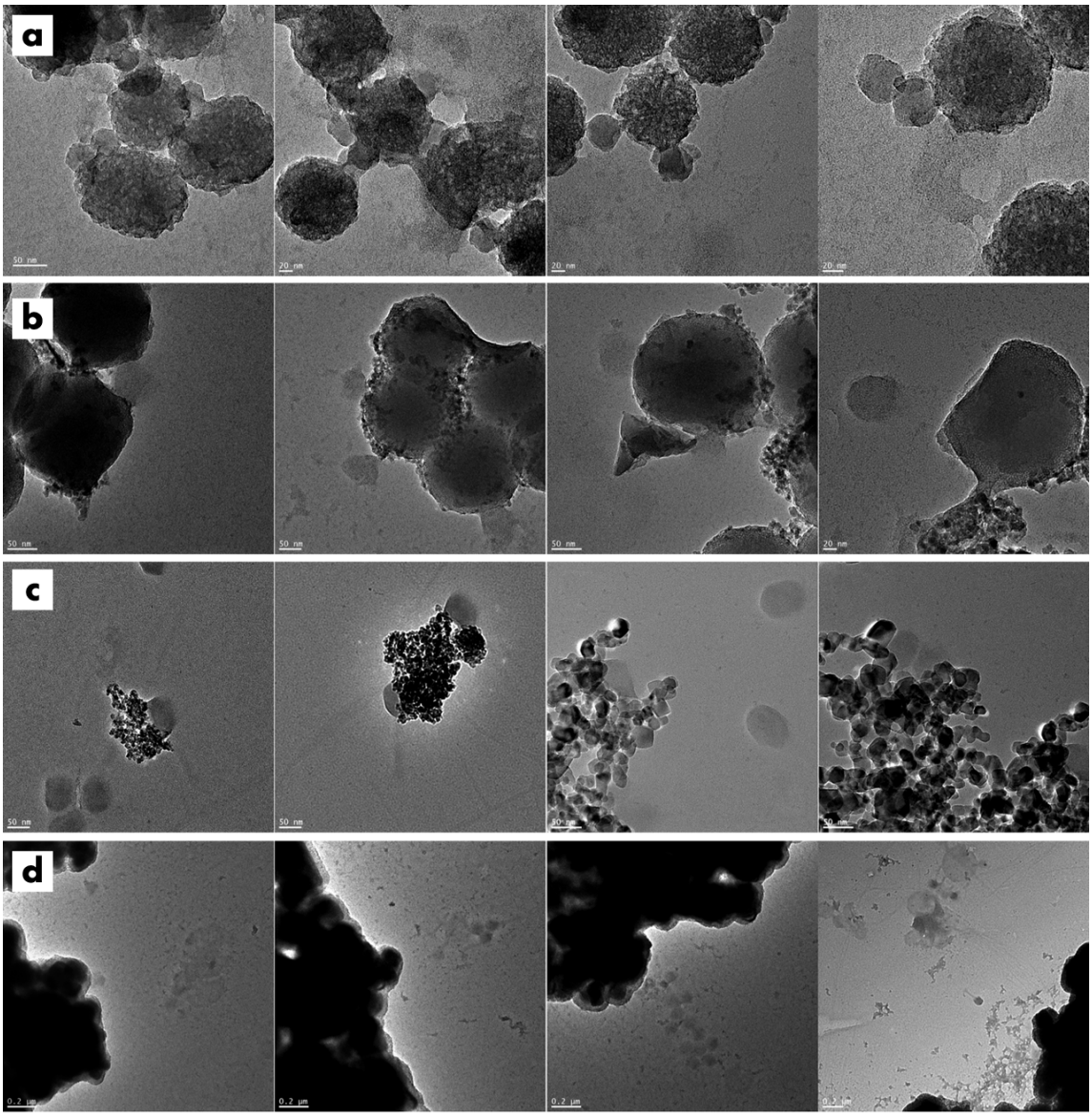

Figure S4. Additional TEM micrographs of phage-nanoparticles interactions (**a – d**). SiO_2 and phage (**a**), SiO_2-TiO_2 and phage (**b**), TiO_2 and phage (**c**), SiO_2-Fe_3O_4-TiO_2 and phage (**d**).

CNT-TiO_2 synthesis method and visualization of the interaction with the phage

For additional interactions visualization test, carbon nanotubes functionalized with titanium dioxide and phage was photographed. Firstly, the nanomaterial was synthesized. Carbon nanotubes

were purchased from Shenzhen Nanotech Port Co, (Shenzhen Nanotech Port Co, Shenzhen, China). Nanotubes modified with titanium dioxide (CNT-TiO_2) were prepared by using concentred titanium (IV) butoxide as a source of titanium dioxide. In order to obtain CNT-TiO_2, 20 mg of CNT previously functionalized with carboxyl groups was added to 1 mL concentrated solution of TBT. Then, the TBT and CNT were sonicated for three hours at the temperature of 50 °C. After sonication, the material was diluted with propanol and centrifuged (8000 rpm for 20 min) to remove the free titanium dioxide precursor. After washing the sample several times with propanol, the material was finally washed with ethanol. The TBT on the carbon nanotubes, exposed to the ethanol hydrolyzed to titanium dioxide. Finally, the sample was heated in airflow at 400 °C for 2 h. Then, according to the method described in the materials and methods section (2.*8. Visualization of phage-nanoparticles interactions*), nanoparticles and phages interactions were visualized (Figure S5). CNT-TiO_2 nanoparticles were also tested for their zeta potential values (40.3 ± 2 [mV]).

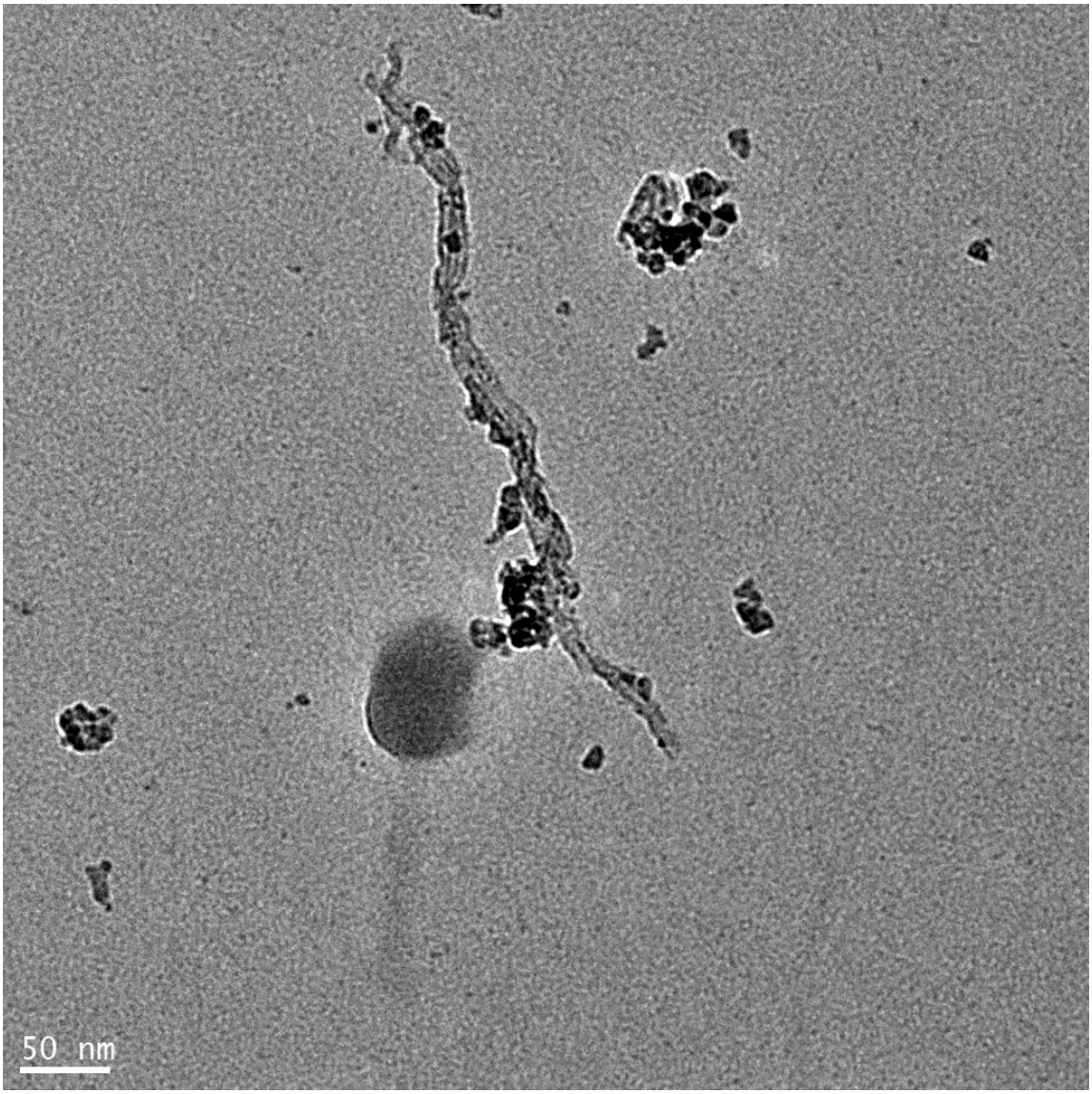

Figure S5. TEM micrograph of CNT-TiO_2 and $T4_5$ phage interaction.

Supplementary materials include: Results from the X-ray diffraction patterns of synthesized SiO_2-Fe_3O_4-TiO_2 particles (Figure S1: X-ray diffraction spectra of SiO_2-Fe_3O_4-TiO_2 particles with marked peaks corresponding to the Fe_3O_4 and TiO_2 phases); Exemplary Petri plate from the coincubation test (Figure S2: Detailed visualization of the results plate from the coincubation test); *E. coli* lysis curves of the tested phage and nanoparticles (Figure S3: Lysis profile experiments of phage $T4_5$ infection in the presence of nanoparticles at different concentrations (0.5, 0.1 and 0.05 mg/mL)); Detailed statistical results of the phage eclipse periods test (Table S1: Dunnett's multiple comparisons test for significance interpretation of the results of progeny phage numbers, from the test of phage eclipse periods after nanoparticles exposure); Additional TEM images of the phage and nanoparticle physical attachments (Figure S4: Additional TEM micrographs of phage–nanoparticle interactions (a–d). SiO_2 and phage (a), SiO_2-TiO_2 and phage (b), TiO_2 and phage (c), SiO_2-Fe_3O_4-TiO_2 and phage (d)); CNT-TiO_2 synthesis method and visualization of the interaction with the phage (Figure S5: TEM micrograph of CNT-TiO_2 and $T4_5$ phage interaction).