Supplementary Information

Broad-spectrum nanoparticles against bacteriophage infections

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Calculation of the molecular mass of MUS:OT (85:15) nanoparticles

The calculations of molecular mass of nanoparticles were performed as previously described in the literature.[1] Nanoparticles are composed of gold core and shell of ligands arranged onto gold surface.

$$M_{tot} = M_{core} + M_{shell}$$

$$V_{core} = \frac{4}{3}\pi(r_{core})^3$$

$$V_{shell} = \frac{4}{3}\pi(r_{core} + r_{shell})^3 - \frac{4}{3}\pi(r_{core})^3$$

$$M_{tot} = \rho_{Au}V_{core} + \rho_{ligands}V_{shell}$$

$$M_w = M_{tot}N_A$$

where M_{tot} is total mass of single nanoparticle, M_{core} is mass of the gold core, M_{shell} is mass of the ligand shell. V_{core} is volume of the gold core, V_{shell} is volume of the ligand shell and N_A is Avogadro number. Radius of gold core r_{core} is 1.35 nm and the length of the MUS ligand r_{shell} equals approximately 1.7 nm. Density of gold ρ_{Au} is 19.3 g mL^{-1} and density of ligands $\rho_{ligands}$ is roughly 1.2 g mL^{-1}. Therefore, molecular mass of nanoparticles is 198.3 kDa.

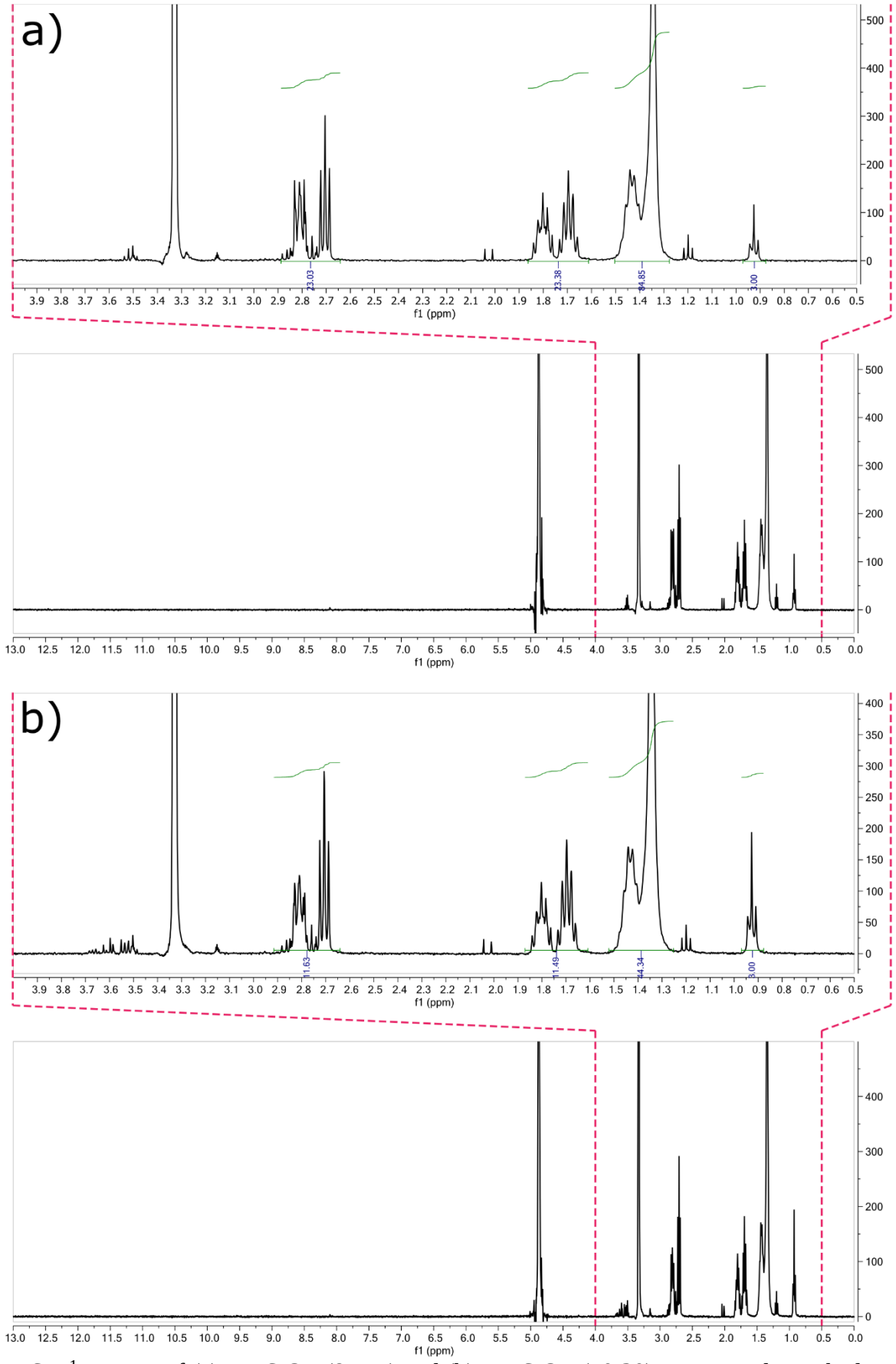


Fig. S1. ^{1}H NMR of (a) MUS:OT (85:15) and (b) MUS:OT (70:30) nanoparticles etched with iodine. Integrated peaks were used to calculate the ratio between MUS and OT ligands as reported by Guven et al.[2] Calculated values were rounded to 5%.

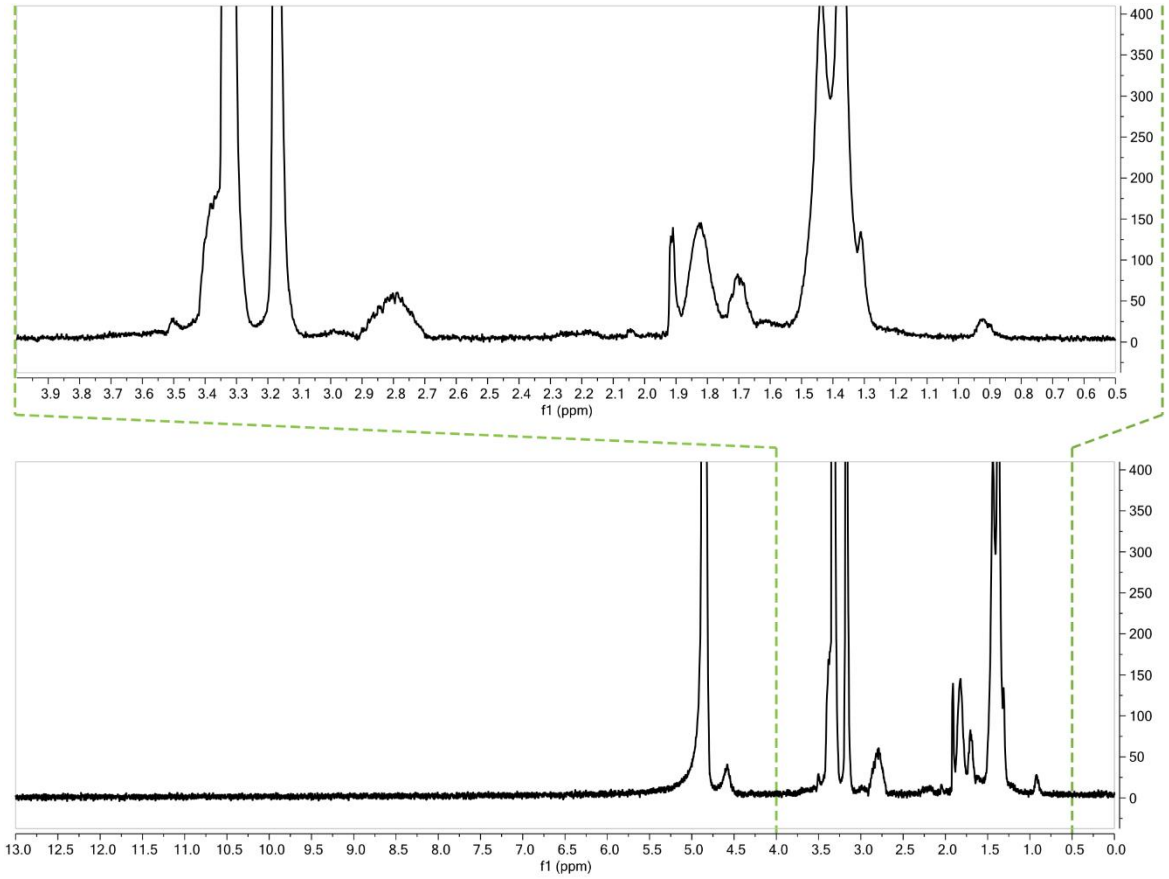
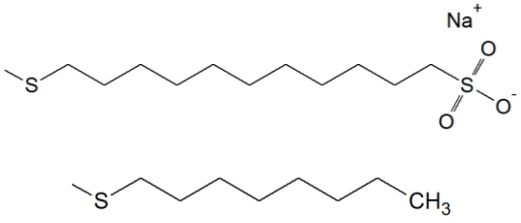
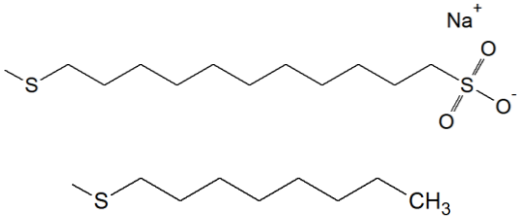
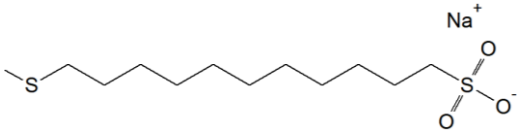
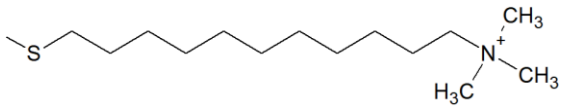
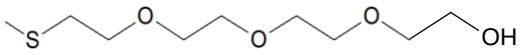


Fig. S2. ^{1}H NMR of TMA nanoparticles etched with iodine.

Table S1. Comparison of properties of all studied nanoparticles.

Name	Activity	Ligands	Core size [nm]	Zeta potential [mV]
MUS:OT (70:30)	Virucidal		3.0 ± 1.2	-19.8 ± 0.8
MUS:OT (85:15)	Virucidal		2.7 ± 1.0	-26.6 ± 3.7
MUS	No effect		3.0 ± 1.0	-

TMA	Virucidal		4.3 ± 1.9	25.6 ± 2.3
EG_4	No effect		3.7 ± 1.1	-13.8 ± 2.3

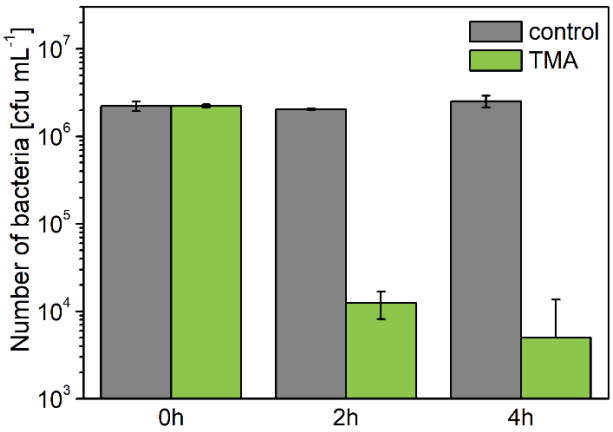


Fig. S3. Analysis of the cytotoxic effect of nanoparticles coated with TMA ligand. Bacteria *E. coli* BL21 were incubated at 37 °C in PBS buffer with 0.5 mg mL^{-1} of TMA nanoparticles. 2 log decrease of alive bacteria was visible after 2 h of incubation. The number of bacteria was measured with the colony count method on LB-agar plates.

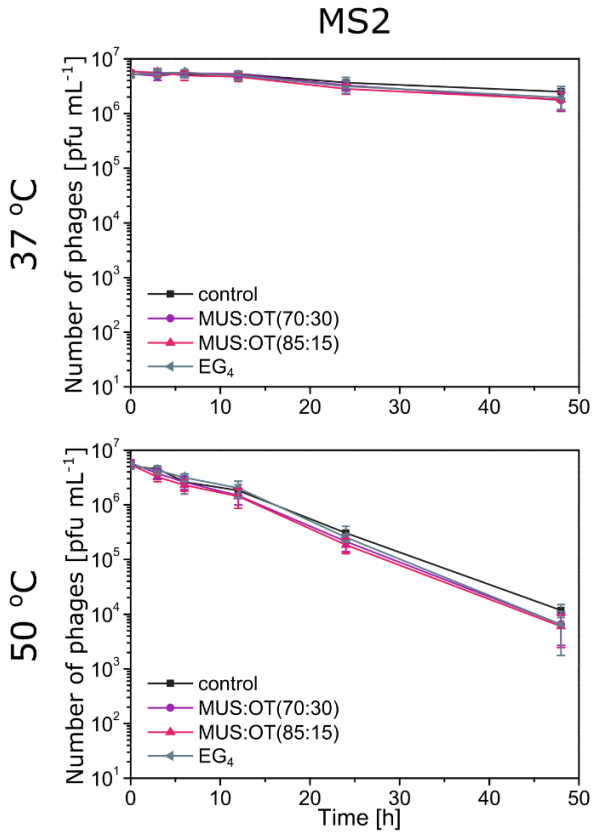


Fig. S4. Analysis of the effect of studied nanoparticles on spherical bacteriophages MS2. Phages were incubated over many days with 0.5 mg mL^{-1} of negatively charged (MUS:OT) and neutral (EG_4) nanoparticles at 37 °C or 50 °C. The number of phages was measured at various time points. Tested nanoparticles showed no deactivating effect against phages MS2.

Synthesis of nanoparticles coated with MUS ligand

First, gold salt ($HAuCl_4 \cdot 3H_2O$; 0.45 mmol) and MUS ligand (0.45 mmol) were dissolved in ethanol (100 mL) and mixed for 15 min. Then, sodium borohydride ($NaBH_4$; 25 mmol) dissolved in ethanol (100 mL) was added dropwise to the mixture upon mixing over 2 h. The reaction was continued for an additional 24 h. Precipitated nanoparticles were then washed by centrifugation using ethanol and finally purified with Milli-Q water on Amicon® Ultra-15 centrifugal filter devices (10k or 30k NMWL). The particles were then suspended in a small amount of water (~2 mL) and freeze-dried.

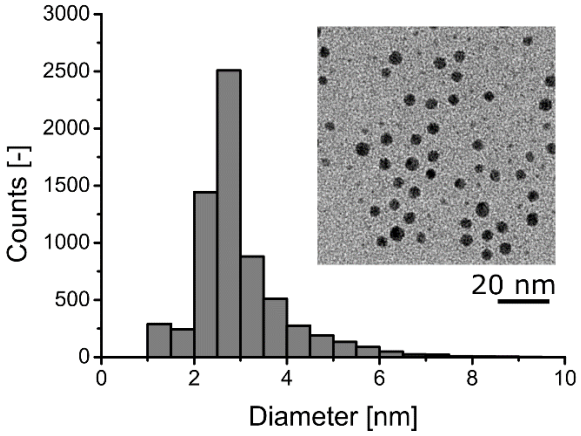


Fig. S5. The size distribution of prepared nanoparticles coated with MUS ligands. Inset shows a representative TEM image.

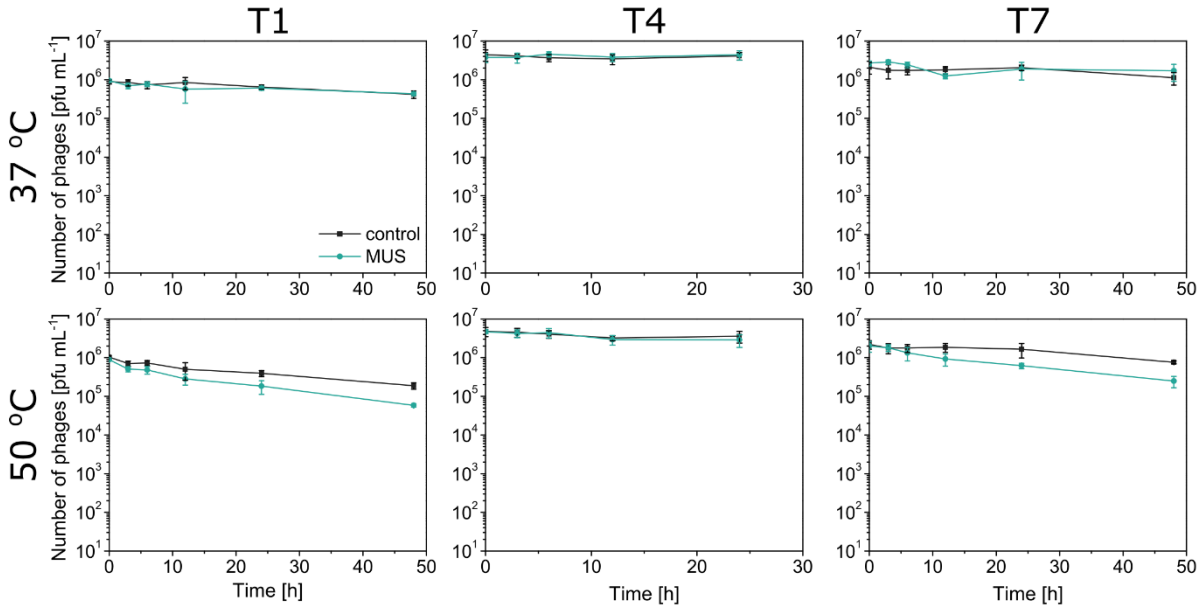


Fig. S6. Analysis of the effect of MUS nanoparticles on bacteriophages T1, T4, and T7. Three types of phages were incubated over many days with 0.5 mg mL^{-1} of MUS nanoparticles at 37 °C or 50 °C. The number of phages was measured at various time points. Studied nanoparticles showed no deactivating effect against any of the three types of phages.

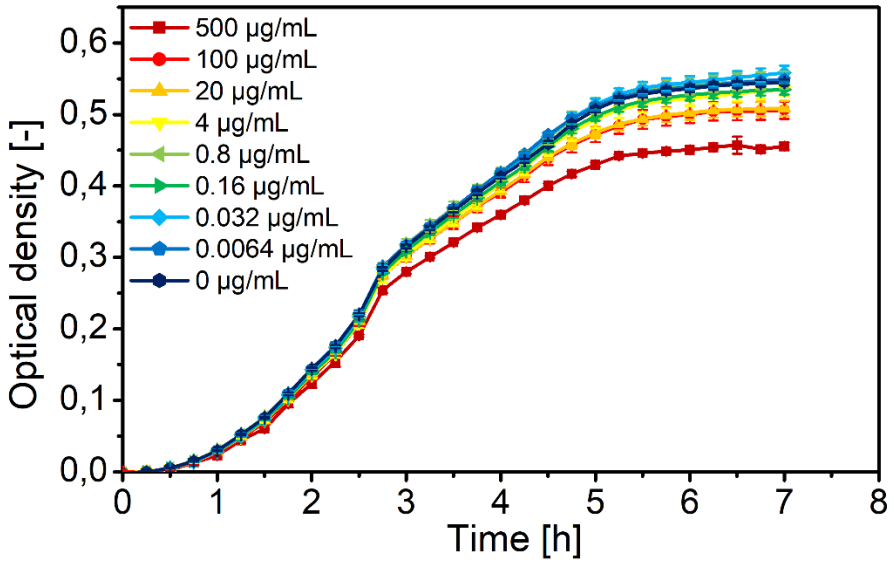


Fig. S7. Influence of nanoparticles MUS:OT (85:15) on bacteria *E. coli* BL21. Growth of bacteria was monitored by measurement of optical density OD_{600}. Obtained results indicate that nanoparticles are not toxic for bacteria in tested concentration range, i.e., $EC_{50} > 500$ µg mL^{-1}.

Table S2. Properties of structural proteins of bacteriophage T4. Only proteins exposed to the external environment were taken into consideration. FASTA sequences of proteins were taken from Uniprot database. ExPASy ProtParam Tool was used to calculate pI values.

Unitprot ID	Protein	Gene	pI [-]	Negatively charged residues (Asp + Glu)	Positively charged residues (Arg + Lys)
CAPSH_BPT4	Major capsid protein	gp23	5.45	46	40
PORTL_BPT4	Portal protein	20	5.36	90	73
CAPSP_BPT4	Capsid vertex protein	24	4.71	60	41
SOC_BPT4	Small outer capsid protein	soc	6.07	11	10
HOC_BPT4	Highly immunogenic outer capsid protein	hoc	4.74	40	26
BP09_BPT4	Baseplate protein gp9	9	5.01	33	24
BP10_BPT4	Baseplate wedge protein gp10	10	4.42	83	43
BP11_BPT4	Baseplate wedge protein gp11	11	5.13	21	15
FIB12_BPT4	Short tail fiber protein gp12	12	5.80	48	43
COMPL_BPT4	Tail completion protein gp15	15	4.88	38	27
TSP_BPT4	Tail sheath protein	18	4.92	74	59
FIBP_BPT4	Long-tail fiber proximal subunit	34	5.29	139	120

FIB35_BPT4	Long-tail fiber protein gp35	35	5.06	36	29
FIB36_BPT4	Long-tail fiber protein gp36	36	7.77	17	18
FIB37_BPT4	Long-tail fiber protein gp37	37	8.54	85	89
WAC_BPT4	Fibritin	wac	4.56	58	36
NECK1_BPT4	Neck protein gp13	13	5.02	36	27
NECK2_BPT4	Neck protein gp14	14	4.57	39	22

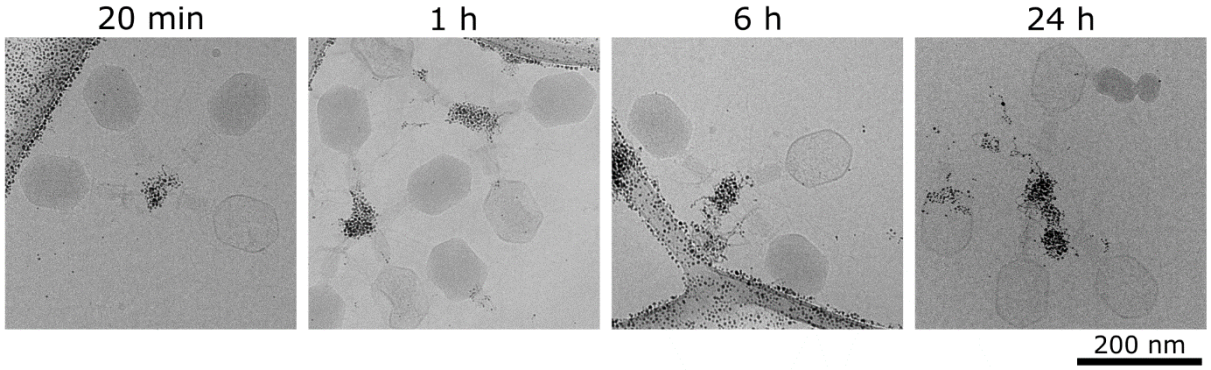


Fig. S8. Time-dependent cryo-TEM analysis of interactions between T4 phages and MUS:OT (85:15) nanoparticles. 4×10^{10} pfu mL^{-1} of bacteriophages were incubated with 0.1 mg mL^{-1} of nanoparticles in TM buffer at 37 °C for different time periods. Then, the cryo-TEM analysis was performed. Interaction between negatively charged nanoparticles and positively charged fibers of phages was visible already after 20 min of incubation.

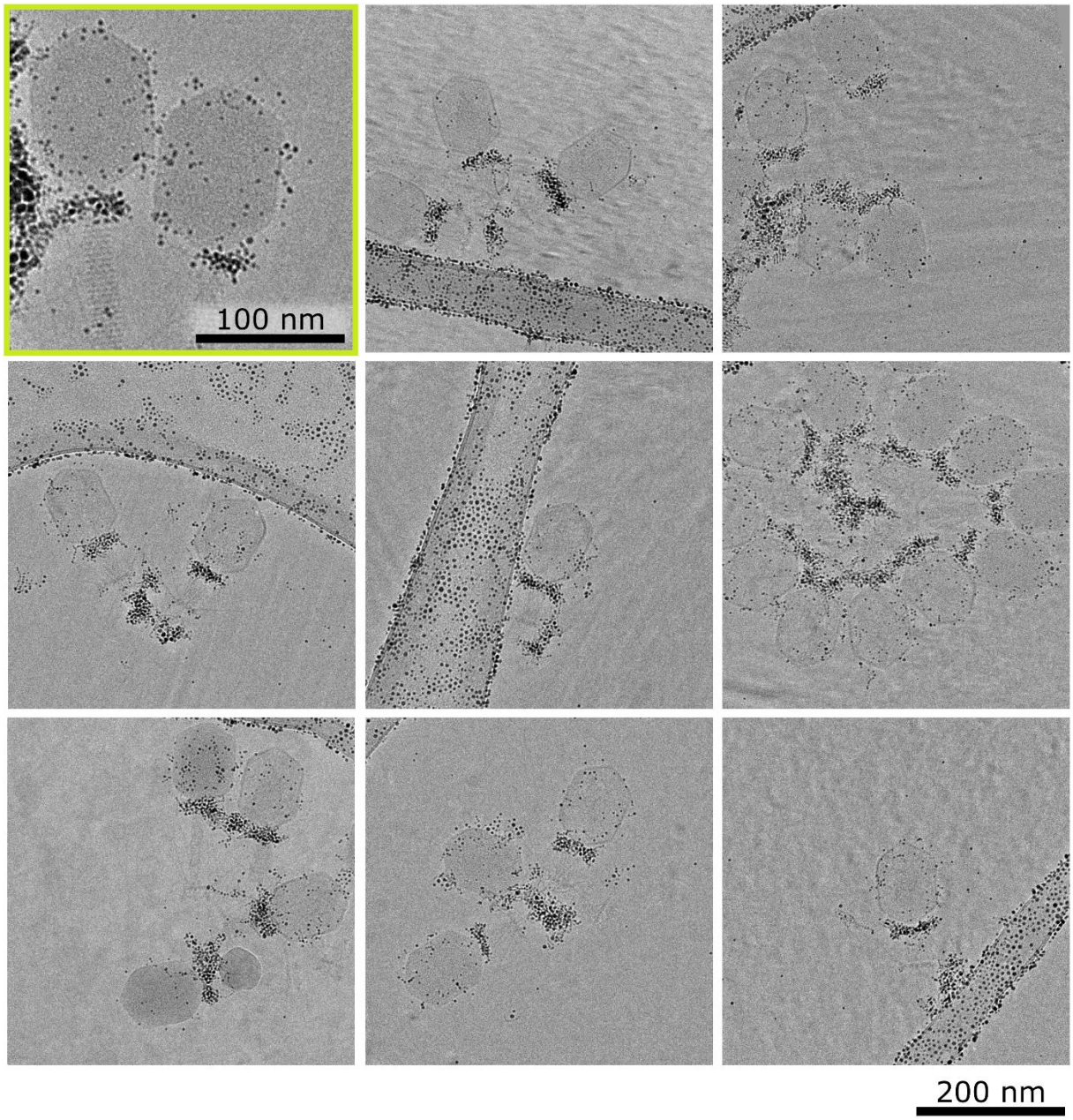


Fig. S9. CryoTEM analysis of interaction between T4 phages and 0.1 mg mL^{-1} of MUS:OT (85:15) nanoparticles at 50 °C for 24 h. Green box indicates the image with different magnification.

References

1 V. Cagno, P. Andreozzi, M. D'Alicarnasso, P. J. Silva, M. Mueller, M. Galloux, R. Le Goffic, S. T. Jones, M. Vallino, J. Hodek, J. Weber, S. Sen, E. R. Janecek, A. Bekdemir, B. Sanavio, C. Martinelli, M. Donalisio, M. A. R. Welti, J. F. Eleouet, Y. Han, L. Kaiser, L. Vukovic, C. Tapparel, P. Král, S. Krol, D. Lembo and F. Stellacci, *Nat. Mater.*, 2018, **17**, 195–203.

2 Z. P. Guven, P. H. J. Silva, Z. Luo, U. B. Cendrowska, M. Gasbarri, S. T. Jones and F. Stellacci, *J. Vis. Exp.*, 2019, **2019**, 1–11.