Supporting Information for

Congo red protects bacteriophages against UV irradiation and allows for the simultaneous use of phages and UV for membrane sterilization

1. *Chemicals used for the experiments*

Congo red (disodium 4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalen-2-yl)diazenylphenyl]phenyl] diazenyl-naphthalene-1-sulfonate), crystal violet (4-{Bis[4-(dimethylamino)phenyl]methylidene}-*N*,*N*-dimethylcyclohexa-2,5-dien-1-iminium chloride), eriochrome black T (sodium 1-[1-Hydroxynaphthylazo]-6-nitro-2-naphthol-4-sulfonate), methylene orange (sodium 4-{[4-(dimethylamino)phenyl]diazenyl}benzene-1-sulfonate), thymol blue (3,3-Bis[4-hydroxy-2-methyl-5-(propan-2-yl)phenyl]-2,1λ^6-benzoxathiole-1,1(3*H*)-dione) and phenol red (phenolsulfonphthalein) were obtained from POCh (Gliwice, Poland). TM buffer contained 10 mM Tris base, 5 µM $CaCl_2$, 10 mM $MgSO_4$, and distilled water (pH=7.4). All components were purchased from Sigma Aldrich (USA). Bacteria were suspended in a 0.9% NaCl (ROTH, Germany). All solutions were sterilized by autoclaving before use. Ultrapure water characterized by the resistivity of 18.2 MΩ·cm was obtained from the Direct-Q water purification system.

LB-agar contained 15 g/l of agar, 10 g/l of NaCl, 10 g/l of tryptone, 5 g/l of yeast extract, and 15 g/l of agar (Carl Roth, Germany), and it was used as an instant mix (Carl Roth, Germany). LB Top-Agar had the same composition, except that the agar concentration was 5 g/l. Liquid LB-medium had the same composition except for lacking 15 g/l of agar (Carl Roth, Germany).

BHI-agar contained 10 g/l meat peptone, 5 g/l bovine heart extract, 12.5 g/l bovine brain extract, 5 g/l sodium chloride, 2.5 g/l dipotassium phosphate, 2 g/l glucose, and 15 g/l agar. Liquid BHI medium had the same composition except for the lack of 15 g/l of agar.

YPD-agar contained 20 g/l casein extract, 10 g/l yeast extract, 20 g/l glucose, and 15 g/l agar (Carl Roth, Germany). Liquid YPD medium had the same composition except for the lack of 15 g/l of agar.

2. *Bacteriophage stabilization against 1 hour of exposure to the UV radiation*

The experiments were conducted according to the same protocol as described in the main text regarding the exposition of the phages to UV radiation for 1 minute. Such a relatively short time was adequate for the experiments' purposes, causing a complete inactivation of phages (control sample, without CR). Knowing that Congo red-protected phages were exposed to around 360 mJ/cm^2. This is the total dose of UV radiation delivered within 1 minute (6 mJ/(cm2·s)). We aimed to verify whether CR is efficient against much higher doses. Results showing protection against 60 times higher doses (i.e., 1 hour of irradiation) are presented in **Figure S1**. Fewer phages survived one hour compared to one-minute irradiation, as expected. However, the decrease in titer for all non-enveloped phages (T1, T4, T7, LR1_PA01, MS2, M13) still did not exceed 2log.

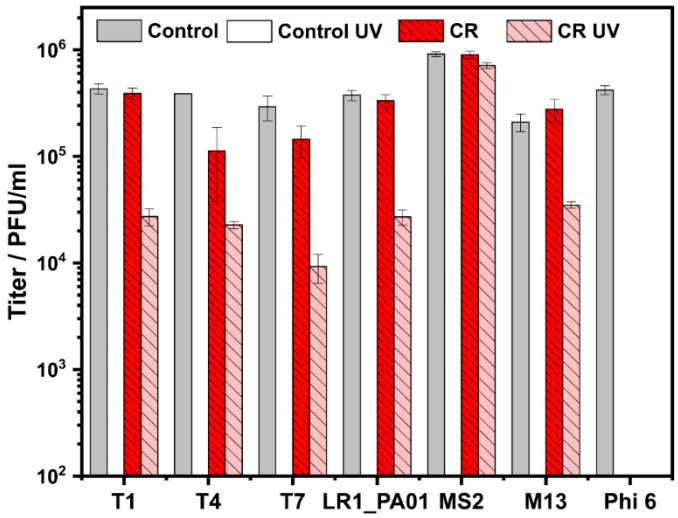

Figure S1. The graph shows the decrease in bacteriophage titers upon 1-hour exposure to UV light. The protective properties of Congo red were examined for seven different bacteriophages T1, T4, T7, LR1_PA01, MS2, M13, and Phi 6. The survival of the phages is presented as a PFU/ml (plague forming units per 1 ml) after 24 hours pre-incubation in 1% Congo red solution and consecutive exposure to the UV (6 mJ/(cm^2·s), 254 nm UV).

3. Bacteriophage stabilization in highly concentrated suspensions

The experiment was conducted according to the same protocol as described in the main text regarding the exposition of the phages to UV radiation for 1 minute. Such a relatively short time was adequate for the experiments' purposes, causing a complete inactivation of phages (control sample, without CR). Knowing that Congo red protected exposed phages in concentrations of about 10^5 PFU/ml, we aimed to verify whether CR is efficient when the concentration of phages is higher. For this purpose, we used the suspensions of all seven examined phages in concentrations of 10^8 PFU/ml, 10^7 PFU/ml, and 10^6 PFU/ml. Results are presented in **Figure S1**. Similar to the experiment presented in **Figure 1A**, in the case of all examined concentrations, the decrease of titer was about 1log when CR was present. Without CR, the decrease to below the detection limit (~25 PFU/ml) was observed regardless of the initial phage concentration. This suggests that 1 minute of UV exposure is enough to cause the decrease in phage titer by at least 8log.

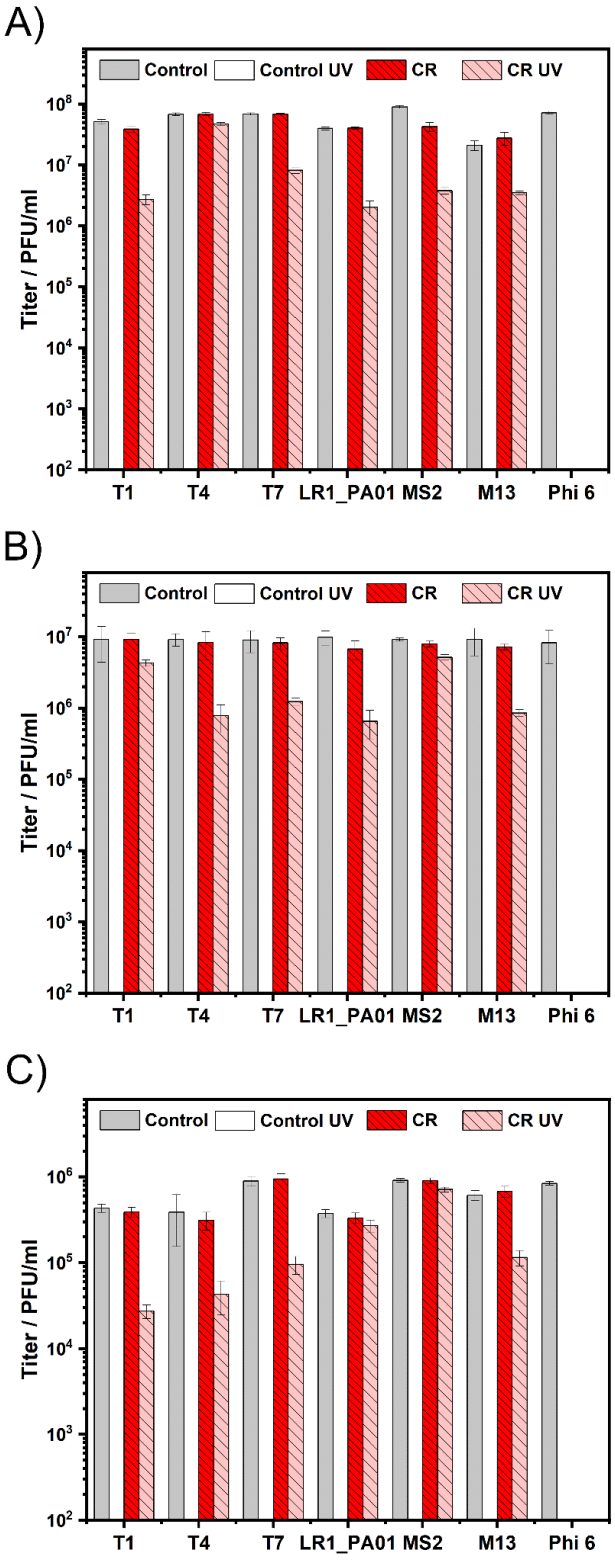

Figure S2. The graph shows the decrease in bacteriophage titers upon 1 min exposure to UV light. The protective properties of Congo red were examined for seven different bacteriophages T1, T4, T7, LR1_PA01, MS2, M13, and Phi 6. The survival of the phages is presented as a PFU/ml (plague forming units per 1 ml) after 24 hours pre-incubation in 1% Congo red solution and consecutive exposure to the UV (6 mJ/(cm^2·s), 254 nm UV). The concentrations of phages were about A) 10^8 PFU/ml, B) 10^7 PFU/ml, and C) 10^6 PFU/ml.

*4. **The effect of SDS on bacteriophages***

10 mg of sodium dodecyl sulfate (SDS) was dissolved in 1 ml of TM buffer, reaching a final concentration of SDS = 1% (w/v). We exposed the representatives of non-enveloped (T4) and enveloped (Phi 6) bacteriophages to SDS at final concentrations of 1% and 0.1%. As the host for the T4 phage, we used *Escherichia coli* BL21 strain, whereas, for Phi 6 phage, we used *Pseudomonas syringae* DSM 21482 strain. Examined phages were diluted in the TM buffer to reach the initial concentration of 10^5 PFU/ml (plaque-forming unit/milliliter). Bacteriophages were diluted in the TM buffer with and without adding SDS to reach the same concentration. The prepared specimens were incubated at 4°C for 24 hours.

Titration was performed by a droplet test on double-layer LB-agar plates. The top-agar layer contained host cells (*E. coli* BL21 for T4 phages or *P. syringae* DSM 21482 strain for Phi 6). After the incubation, a droplet test was performed by placing at least eight droplets of phage suspension on the top-agar layer. The experiment was performed in triplicate.

Phi 6 phage was incapable of surviving in neither 0.1% nor 1% (w/v) concentration of SDS. T4 phage was still infective after the incubation in SDS. Results are presented in **Figure S1.**

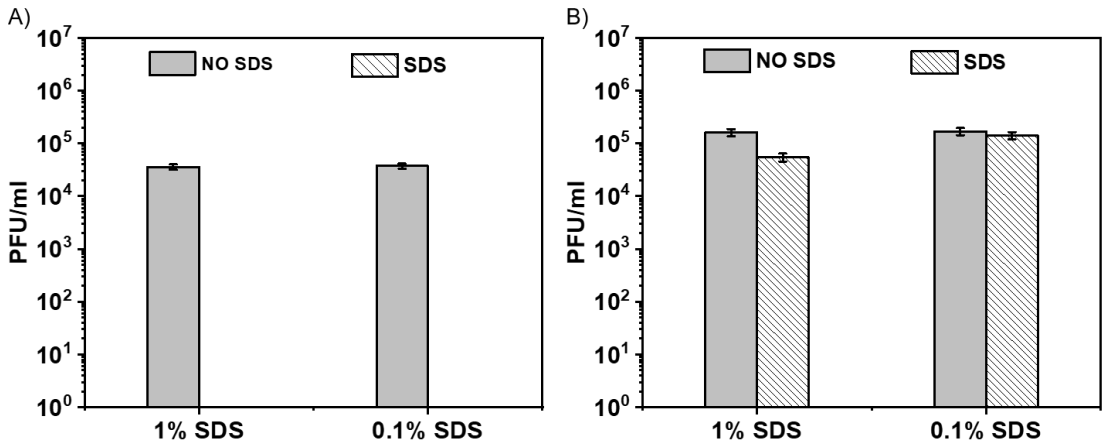

Figure S3. The comparison of the effect of incubation in sodium dodecyl sulfate solution on the representatives of A) enveloped (Phi 6) and B) non-enveloped (T4) bacteriophages.

According to the literature, the lipid envelope is essential for all enveloped viruses to remain infective [1]. The damage or destabilization of its envelope results in the inactivation of the virus. The lipid envelope can be destabilized by the effect of detergents (surfactants) [2] or ethanol [3]. It is also known that Congo red has mild detergent-like properties [4]. These properties explain why we observed a complete inactivation of Phi 6 bacteriophage in the presence of Congo red event before the exposition to the UV radiation (**Figure 1A**). Comparing these results with the effect of a regular surfactant, SDS, on Phi 6 phage

reassured us that the same phenomenon is responsible for the inactivation of enveloped viruses during the experiments. The schematic illustration of this process is presented in **Figure S2**.

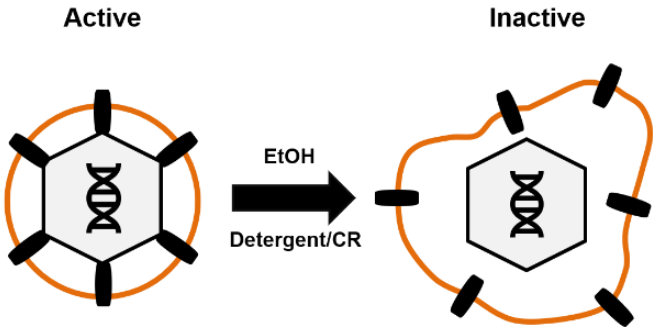

Figure S4. The mechanism of inactivation of enveloped viruses is proposed based on the experiments on Phi 6 phage – a surrogate for enveloped eukaryotic viruses. Detergent-like properties of Congo red dye cause the destabilization of the viral lipid envelope, which is essential for the infectivity of enveloped viruses. The same effect is observed for detergents (e.g., SDS) or ethanol [3].

5. ***Congo red and dyes absorption of the UV-Vis radiation***

We observed significant changes in Congo red UV-Vis spectra both in time and upon UV irradiation. First, we analyze the changes in time. We compared the Congo red solutions in 3 different solvents: ultrapure water (Type-I, Direct-Q) (**Figure S5A**), PBS buffer (**Figure S5B**), and TM buffer (**Figure S5C**). Spectra were recorded every 5 minutes for 60 minutes.

CR solutions in water and PBS were stable, and UV spectra did not change significantly (**Figure S5A** and **S5B**). Concentrated samples of CR in TM buffer (e.g., 0.5 mg/ml) were turbid, in contrast to ultrapure water solutions of the same concentration. We observed partial precipitation of reddish sediment in TM buffer in diluted samples. The absorbance of CR in the TM buffer decreased over time, and the maximum absorbance shifted from 490 nm to about 580 nm while the spectrum changed its shape (**Figure 3B**). The explanation for this is provided in the main text.

TM caused a significant change in the shape of CR spectra within about 25 minutes after the preparation. We recorded UV-Vis spectra of CR in water spiked with separate TM buffer components, namely 5 µM $CaCl_2$ (**Figure S5D**), 10 mM $MgSO_4$ (**Figure S5E**), and 10 mM Tris (**Figure S5F**). This experiment proved that Mg^{2+} cations were responsible for the changes in the shape of the Congo red spectrum. Mg^{2+} cations are necessary for the number of phages to function correctly [5].

We also redid the experiments shown in the main text in **Figure 3**, but in PBS, instead of TM buffer. Results are shown in **Figure S5G**, **H**, and **I**.

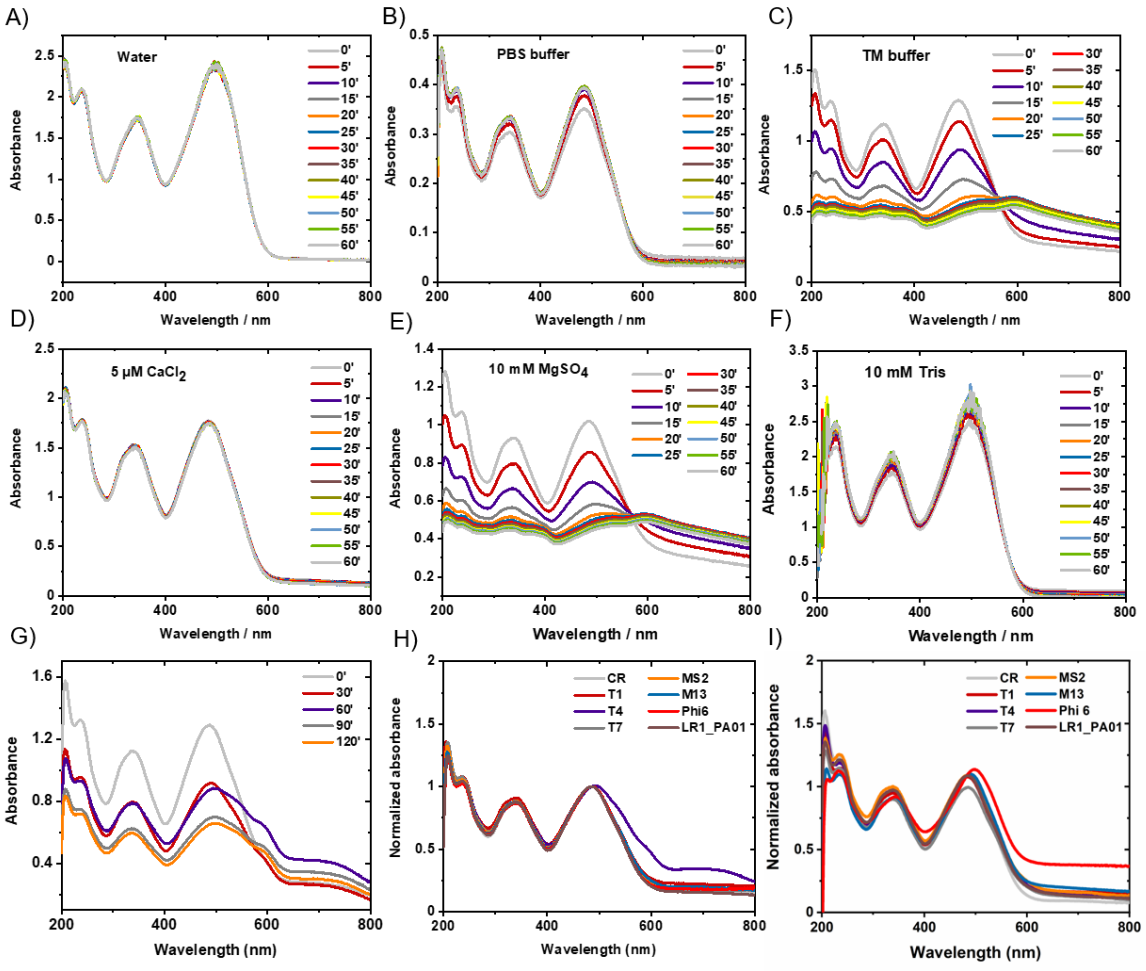

Figure S5. The UV-Vis spectra of the CR with the presence of different ions. Measurements were conducted every 5 minutes in the solutions of A) Direct-Q deionized water, B) PBS buffer, C) TM buffer, and Direct-Q deionized water spiked with separated components of TM buffer: D) 5 µM $CaCl_2$, E) 10 mM $MgSO_4$, and F) 10 mM Tris-base. G) The degradation of CR in PBS buffer due to UV irradiation (254 nm + 365 nm; 8W). The solution was incubated for 1 hour before the irradiation. H) UV-Vis spectra of 1% CR in PBS buffer with T1, T4, T7, M13, Phi 6, MS2, and LR1_PA01 bacteriophages. The measurements were recorded after 1-minute incubation of bacteriophages in CR. Spectra were normalized to 483 nm. I) UV-Vis spectra of 1% CR in PBS buffer, with T1, T4, T7, M13, Phi 6, MS2, and LR1_PA01 bacteriophages. Measurements were performed after 24 hours of incubation of bacteriophages in CR. Spectra were normalized to 586 nm. A slight shift in the maximum absorption (~ 480 nm → ~ 490 nm) was observed in both H) and I).

1% solutions of five other dyes (eriochrome black T, crystal violet, methylene orange, thymol blue, and phenol red) were investigated for their potential UV-protective properties. Only Congo red protected phages from UV irradiation (**Figure 1D**).

We recorded the UV-Vis spectra of these dyes and compared them with CR. Freshly prepared 1% solutions of all dyes (including CR) were diluted 500 times, so the absorbance value was between 1 and 2. The measurements were performed in PBS (**Figure S6A)** and TM buffer (**Figure S6B)**.

The spectra analysis showed that all examined dyes absorb the radiation in the UV-C (254 nm). The specific irradiation wavelength was marked with the black vertical line in **Figure S6**. These dyes should protect bacteriophages against UV. However, only CR did. This was most likely due to the specific interactions between CR molecules and proteins, proved in microbiology for staining [6].

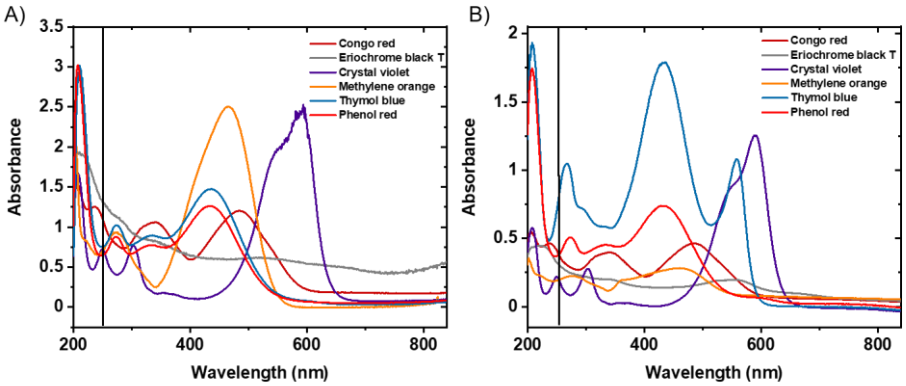

Figure S6. The UV-Vis spectra of Congo red, eriochrome black T, crystal violet, methylene orange, thymol blue, and phenol red in A) PBS and B) TM buffer. The black vertical line corresponds to the wavelength of 254 nm, i.e., one used in phage-related experiments.

To compare the spectra shift in mixtures of Congo red and phages, we prepared fresh (used about 1 minute after preparation) 1% solutions of Congo red in TM buffer and PBS buffer solution, containing 10 µg/ml of human serum albumin (HSA) (Sigma Aldrich, MA, USA). We expected to observe a similar shift in the pure protein solution.

We recorded UV-Vis spectra of fresh CR and HSA mixtures in PBS buffer (**Figure S7A**) and TM buffer solution (**Figure S7B**). As a control, we used CR solution in these buffer solutions. Before the measurement, solutions were diluted 500 times, so the absorbance value was between 1 and 2.

The spectra analysis showed a spectra shift of about 490 nm → 500 nm in both buffer solutions when HSA was present. This shift is caused by forming a complex of HSA protein and CR. According to the literature, such shifts were also observed for bovine serum albumin (BSA). This complexation phenomenon was proven using spectroscopic techniques, including UV-Vis spectroscopy, fluorescent spectroscopy, and circular spectroscopy dichroism [7,8]. It is also known that in aqueous solutions, the form of serum albumins complexed with CR is preferred from the thermodynamics point of view [9].

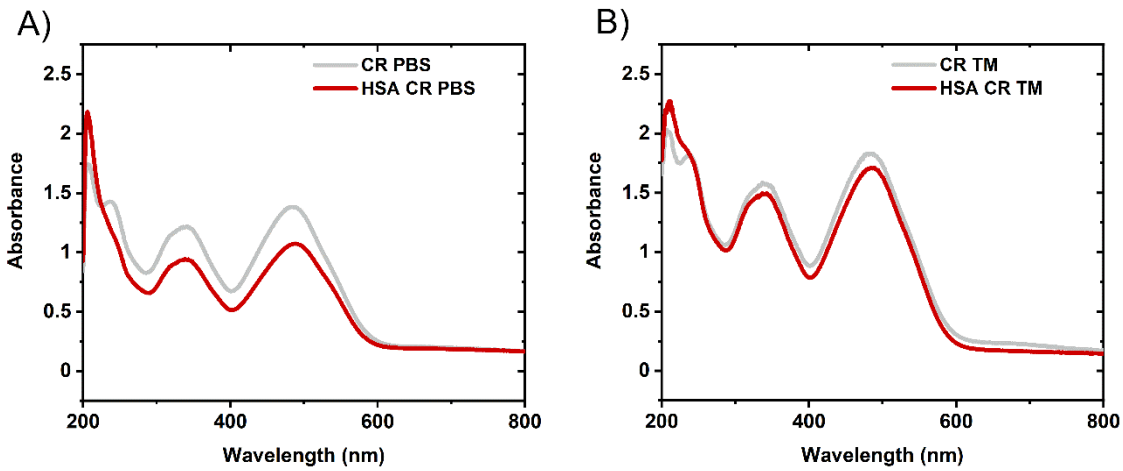

Figure S7. The UV-Vis spectra of Congo red with human serum albumin (HSA) in A) PBS and B) TM buffer.

6. *Mechanisms of Congo red inactivation of enveloped phages and UV-protection of non-enveloped phages*

Free radicals generated upon UV irradiation cause viral genome damage and deactivation of virions. CR molecules absorb most of the UV radiation, limiting the dose to reach virions and lowering the number of inactivated viruses. The principle of the "microbial shield" mechanism is that a high dye concentration in the suspension containing viruses absorbs the UV radiation. The "molecular sunscreen" mechanism assumes that CR binds to viral capsid resulting in a local increase in the CR concentration and local protection from UV. Our experimental results (**Figures 1A, 3C-D, S5H-I)** and literature review [10,11] suggest that even though the solution probably absorbs some piece of radiation, the principle of CR protective properties is due to the "molecular sunscreen" mechanism (i.e., requiring binding between dye and virions), schematically illustrated in **Figure 5**.

7. *Estimation of binding constant*

To confirm the interactions of Congo red with bacteriophages, we performed a spectrophotometric titration of 1% Congo red solution in TM buffer with bacteriophage suspension. The initial concentration of T4 bacteriophages in the suspension used for titration was 10^7 PFU/ml. After the preparation of 1 mL of fresh 1% solution of Congo red in the TM buffer, 2 µL portions of bacteriophage suspension (10^7 PFU/ml) were added. Effectively the concentration of phages in the solution grew by 2×10^4 PFU/mL per each portion. After each portion, the sample was diluted 500 times (to provide the absorbance value below 2), and UV-Vis spectrum measurement was provided. Phages were added to reach the final concentration of about 3×10^{10} PFU/mL. The time of the analysis was 5 minutes to eliminate the effects of the presence of Mg^{2+} on the CR spectrum.

We chose to compare the differences in the absorbance at the wavelength 600 nm. At this value, the absorbance of the bacteriophage is neglectable (extinction coefficient $\varepsilon_{T4} \approx 0$). The results were plotted as the correlation between phage concentration and the absorbance at 600 nm wavelength and presented in the figure below (***Supporting Information,*** **Figure S8**):

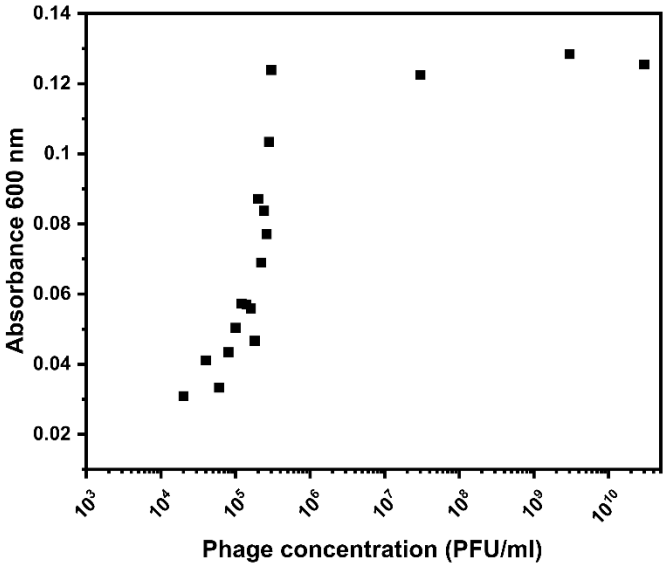

Figure S8. The correlation between T4 phage concentration and the absorbance of 1% CR solution. An increase in absorbance value is observed until reaching the phage concentration of about 3×10^5 PFU/ml; in phage concentrations of 3×10^7, 3×10^9 and 3×10^{10} the absorbance value was similar.

The estimation of the binding constant was done based on the Scatchard method [12] with the modification described by Healy [13]. Binding constant β is defined as:

$$\beta = \frac{[S\ CR]}{[CR][S]}$$

where [CR] is the concentration of the free Congo red in equilibrium, [S] is the concentration of unoccupied binding sites (number of all unoccupied sites at all of the bacteriophages per volume), and [SL] is the concentration of occupied binding sites (N_{oc}) (analogically).

$$N = N_{oc} + N_{unoc}$$

where N – an average number of possible binding sites on a bacteriophage, N_{oc} – an average number of occupied binding sites on a bacteriophage, N_{unoc} – an average number of unoccupied binding sites on a bacteriophage

$$\frac{N_{oc}}{N} = \frac{[S\ CR]}{[S\ CR] + [S]}$$

$$N_{oc} = N\frac{\beta[CR]}{\beta[CR] + 1}$$

The fraction of the ligand bound to the bacteriophage during the titration can be expressed as:

$$f = \frac{A_{obs} - A_{CR}}{A_{max} - A_{CR}}$$

where f stands for the fraction, A_{obs} is the absorbance measured at 600 nm, A_{CR} is the absorbance coming from the free congo red, and A_{max} represents the maximal absorbance.

To improve the quality of the calculation, the experimental values of measured absorbance were fitted by the exponential function with $R^2 \approx 0.88$. The absorbance of free Congo red is almost equal to the total CR concentration at the beginning and almost 0 at the end of the titration. To minimize the error coming from using one of the estimations (A_{CR0} stands for the absorbance of CR before adding the first portion of bacteriophages):

$$f \approx \frac{A_{obs}}{A_{max}} \text{ or } f \approx \frac{A_{obs} - A_{CR0}}{A_{max} - A_{CR0}}$$

We used the function $A_{CR} = A_{CR0} \cdot \left(1 - \frac{[T4]_T}{[T4]_{Tmax}}\right)$, where $[T4]_T$ is the total concentration (all forms of T4 and T4-CR_n) of bacteriophage and $[T4]_{Tmax}$ is the total concentration of the added bacteriophage when $A_{obs} \approx A_{max}$.

$$N_{oc} = \frac{f[CR]_T}{[T4]_T}$$

$$\frac{f[CR]_T}{[T4]_T} = N\frac{\beta[CR]}{\beta[CR] + 1}$$

$$[CR] = [CR]_T(1 - f)$$

Hence:

$$\boldsymbol{\frac{[T4]_T}{f} = \frac{1}{N\beta(1 - f)} + \frac{[CR]_T}{N}}$$

From the linear regression ($R^2 \approx 0.99$)

$$\boldsymbol{\beta = 1.28 \cdot 10^4 \pm 0.09 \cdot 10^4 \frac{dm^3}{mol}}$$

$$\boldsymbol{N = 2.40 \cdot 10^{11} \pm 1.59 \cdot 10^{10}}$$

Obtained N value is surprisingly high. This is, however, in line with the proposed mechanism, where a large number of molecules stick to virions and adsorb UV radiation, acting as "molecular sunscreen".

8. ***Schematic illustration of membrane filter sterilization***

In this work, we used Nylon 66 0.22 µm syringe filters as the membrane model in bioreactors. To simulate bacterial biofouling of the membrane, 5 ml of bacterial suspension in 0.9% saline was filtered through the filter. Since the diameter of filter pores was much smaller than the average size of the bacterial cell, bacteria covered the surface of the membranes (**Figure S9-1)**). Next, membrane filters were filled with 1 ml of TM buffer solution containing bacteriophages, Congo red, or both (**Figure S9-2)**). The volume of 1 ml was found to be enough to fill the syringe filter without going through it. Next, all the filters were kept in the dark for 15 minutes. The following 15 minutes of incubation took place either in the dark or exposure to UV radiation in a laminar hood (Thermo Scientific MSC-ADVANTAGE; 36 W lamp, 254 nm UV) (**Figure S9-3)**). After the incubation, all the filters were reversely washed with 1 ml of fresh TM buffer solution, removing phages, some of the dye, and alive bacteria (**Figure S9-4)**), leaving the membrane sterilized. The entire procedure was schematically illustrated in **Figure S9**.

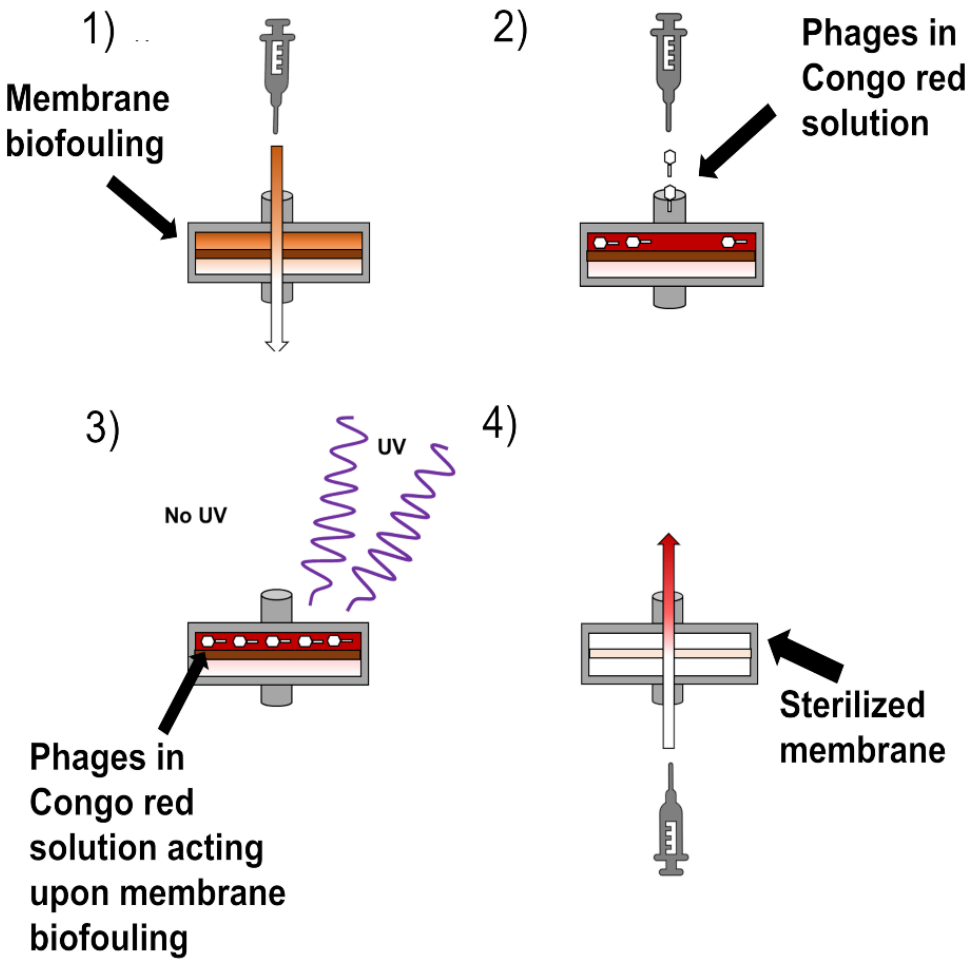

Figure S9. Schematic illustration of membrane sterilization experiment: 1) Bacterial cells are embedded in the syringe filters. 2) Syringe filters are additionally filled with a suspension containing phages and/or CR. 3) Incubation for 30 minutes with/without 15 minutes of exposition to the UV. 4) Filters flushed with 1 ml of fresh 0.9% saline.

9. ***Examination of membrane integrity after the cleaning procedure***

The usage of UV radiation for membrane sterilization is generally recognized as safe for membrane integrity. In the paper by Lee et al**.,** the authors proved the exposure of PVDF membranes to the UV for up to 250 hours affects neither their structure nor chemical composition significantly. However, increased permeate flux and decreased thermal stability were observed [13]. In time UV might introduce some adverse effects on membrane properties. They seem to be acceptable, along with the low turbidity of sewage, for numerous reports describe UV sterilization of membrane bioreactors (MBRs) [14–16]. Our sterilization protocol allows for decreasing the time of UV exposure, which would potentially result in slowing down the appearance of these adverse effects.

To confirm these observations in our experimental design, we exposed Nylon66 and PTFE syringe filter membranes to 15 minutes of UV radiation (360 mJ/cm^2). Afterward, the syringe filters were cut open, and the membranes were visualized using the optical microscope (10x magnification). This was to observe potential damage in membranes' structure caused by UV radiation. For each membrane, three images were taken before (panels A and C) and after the UV exposure (panel B and D). Images are summarized in **Figure S10**.

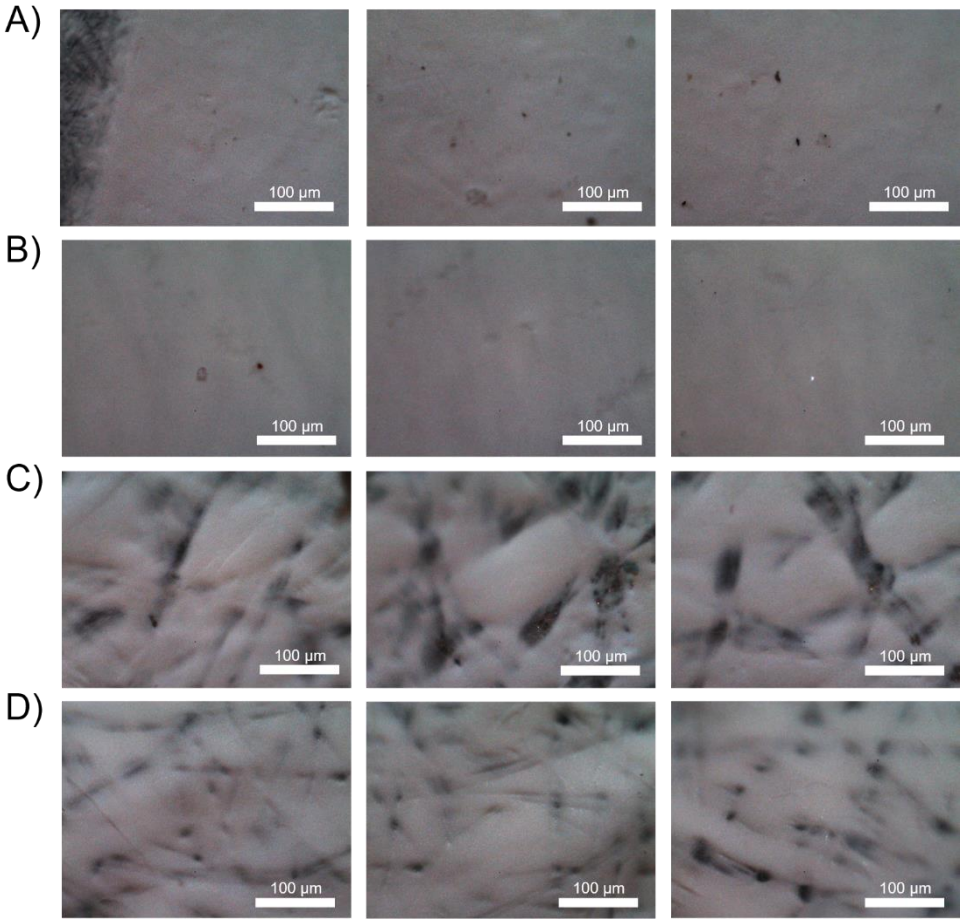

Figure S10. Visualization of Nylon66 and PTFE membranes using an optical microscope (10x magnification). A) Nylon66 membrane before the UV exposure, B) Nylon66 membrane after 30 minutes of UV exposition, C) PTFE membrane before the UV exposure, and D) PTFE membrane after 30 minutes of UV exposition.

With this visualization, we didn't observe any significant differences in membrane surface structure before (A, C) and after 15 minutes of UV exposure (B, D).

10. ***Examination of Congo red penetration through the membranes***

To verify if Congo red penetrates through membranes, potentially affecting the permeate quality, we used PTFE (polytetrafluoroethylene) 0.22 µm syringe filter to simulate the surface of the bioreactor membrane. According to the literature, polyvinylidene difluoride (PVDF) membranes are most frequently used in MBR. We found PTFE as a material of similar properties. Both PTFE and PVDF membranes are hydrophobic, have low protein binding ability, can be used for the filtration of gases and air, and both have good chemical compatibility. PTFE is suggested for the filtration of solutions containing aggressive solutions, e.g., strong acids, while PVDF is more suitable for non-aggressive aqueous and organic solutions (http://en.finetech-filter.com/what-is-the-difference-between-pvdf-and-ptfe.html).

Filters were filled with 1 mL of 1% of Congo red solution in distilled water and incubated at room temperature for 30 minutes (the cleaning procedure described in our manuscript). Then, filters were rinsed multiple times with 1 mL of distilled water. After each rinsing, the solution was transferred to the glass vial, and UV-Vis spectrum measurement was provided. The differences in absorbance are presented in **Figure S11**.

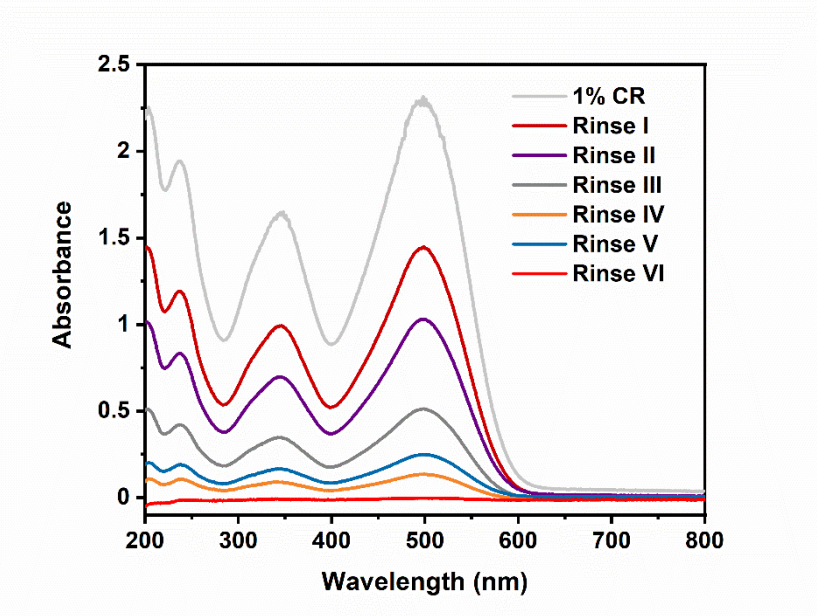

Figure S11. The differences in Congo red absorbance after exposing the PTFE membrane to 1% Congo red solution (1% CR) and after six rinsings. With each rinsing, the decrease in the absorbance value was observed until reaching the background intensity by Rinse VI.

The drop of the Congo red concertation after each rinsing was also observed as the decrease in the intensity of the color, presented in **Figure S12**:

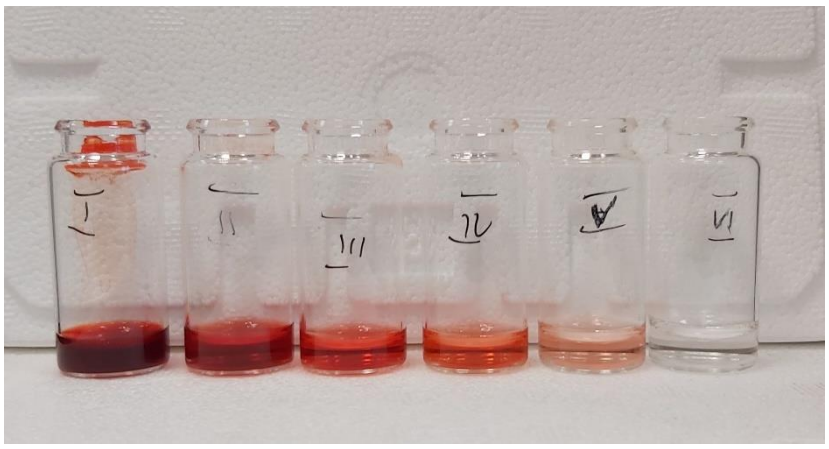

Figure S12. Congo red solutions rinsed from the PTFE membrane. The decrease in color intensity corresponds to the decrease in CR absorbance.

Six risings were required to remove Congo red to below the spectrophotometric assays' detection limits (I – VI). This is in line with our testing experiments, where filters were rinsed slowly with 5 mL of buffer (in total), i.e., the amount that was enough to 'clean' the filter.

Knowing the surface of the syringe filter membrane is about 16 cm^2, for the removal of Congo red from the membrane, 6 mL of water per 16 cm^2 is needed; 3750 mL/1 m^2, so less than 4 L of water per 1 m^2 of the membrane (the cost of 1 L of water is about 0.002$). After rinsing, no signs of Congo red penetration through the membrane were observed. For

a certain complete removal of Congo red, after the cleaning procedure, one of the protocols for Congo red removal from the aqueous solution described in the literature [10–12] might be applied**.**

11. ***Reusability of phage-Congo red solution for membrane sterilization***

We aimed to verify if the "cleaning mixture" (composed of T4 phages and CR) could be reused after the membrane sterilization protocol described in the main text. In short, *E. coli* BL21 (DE3) GFP (of concentration of 3×10^4 CFU/ml) were deposited onto Nylon66 0.22 µm filters by filtering 5 ml of the bacterial suspension in 0.9% NaCl twice through the membrane (**Figure S9 1)**). Next, filters were filled with 1 ml of TM buffer containing T4 (around 10^3 PFU/ml) suspension and CR (0.1% w/v) (**Figure S9 2)**). This volume was previously found to be enough to fill the filters but not go through. Filters were incubated at room temperature for 15 minutes in the dark, then exposed to UV irradiation within the laminar hood (Thermo Scientific MSC-ADVANTAGE; 36 W lamp, 254 nm wavelength) for 15 minutes (**Figure S9 3)**). Finally, the filters were washed with fresh TM to investigate the effect of the treatment (**Figure S9 4)**). This final washing was done in the opposite direction to the previous steps. Such protocol allowed the recovery of most cells from the control samples.

Here, we collected the suspension after the final flushing (1 ml of solution), and we used it as a "cleaning mixture" for consecutive sterilization. Before the second application, the "cleaning mixture" was centrifuged (5 minutes, 2000 rpm) to remove bacterial debris. Centrifuging for a longer time or at a higher angular velocity may cause the sedimentation of the dye. Then the sterilization protocol was repeated on freshly prepared Nylon66 filters spiked with bacteria. Instead of a fresh "cleaning mixture," the used one was applied. In total, three cycles of sterilization were completed. The experiment was conducted in triplicate.

Such protocol proved efficient for two consecutive sterilization runs but not for the third run (**Figure S13**). This is probably due to the decreasing concentration of Congo red, for not all the dye could be retrieved by just washing membranes with a water solution. In the first two cycles, the decrease in bacterial concentration was from 2×10^4 CFU/ml to below the limits of detection (~10 CFU/ml), suggesting the combined effect of UV radiation and bacteriophages protected by Congo red. After the third cycle, this decrease was from 2×10^4 CFU/ml to 2×10^3 CFU/ml, comparable to the sterilization with combined UV radiation and bacteriophages (with no Congo red).

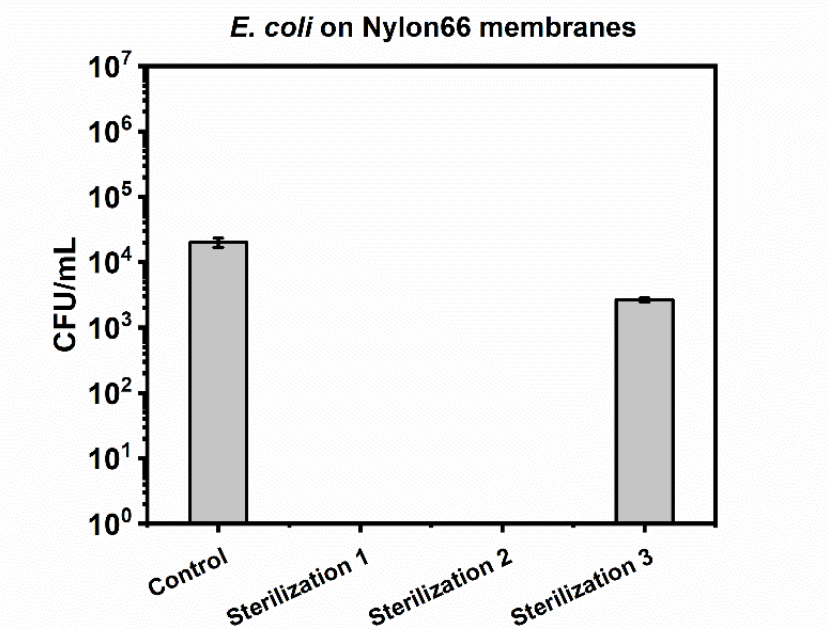

Figure S13. Multiple membrane filter sterilization from *E. coli* BL21 (DE3) GFP biofouling with the same suspension of T4 bacteriophages with CR in TM buffer and simultaneous UV exposure.

12. *Costs estimation*

The cost of 1 kg of Congo red powder is about 1.45$ (when buying in more significant amounts, e.g., https://www.indiamart.com/proddetail/direct-congo-red-21950311862.html). Our protocol requires Congo red concentration of 10 mg/mL → 10 g/L → 1 kg/100 L; the cost of 1L of water is 1.73$ per m^3 (about 0.002$ per 1 L). Congo red used per 1 L of the solution is about 0.01$. Including the cost of consumables for phage amplification – Petri dishes ($0.01 per plate), Luria-Bertani agar (0.07$ per plate), and Luria-Bertani Top agar (0.05$), the total cost of 1 L of the 'cleaning mixture' is about 0.15$. 1 mL of the 'cleaning mixture' is enough to sterilize approximately 10 cm^2 of the membrane. Therefore, 1L covers approximately 100 m^2 of the membrane. The average cost of UV sterilization with a dose of 360 mJ/cm^2 is about 0.63$. Assuming the sterilization cost of a 100 m^2 membrane with UV radiation for 30 minutes is 6300$, the cost of 15 minutes of exposure would be about 3150$. Including the costs of the preparation of the cleaning mixture (0.15$), the estimated total cost of this sterilization method would be almost two times lesser than in the case of just UV exposure. This is due to the shorter time of irradiation.

13. *Supporting Information references*

1 A. Eisenstark, R. L. Buzard and P. S. Hartman, Inactivation of Phage By Near-Ultraviolet Radiation and Hydrogen Peroxide, *Photochem. Photobiol.*, 1986, **44**, 603–606.

2 M. E. Wiebe, A. Lippin and M. H. Stryker, Inactivation of viruses in labile blood derivatives. I. Disruption of lipid-enveloped viruses by tri(n-butyl)phosphate detergent combinations, *Transfusion*, 1985, **25**, 516–522.

3 S. Watts, M. Ramstedt and S. Salentinig, Ethanol Inactivation of Enveloped Viruses: Structural and Surface Chemistry Insights into Phi6, *J. Phys. Chem. Lett.*, 2021, **12**, 9557–9563.

4 C. Lendel, B. Bolognesi, A. Wahlström, C. M. Dobson and A. Gräslund, Detergent-like interaction of congo red with the amyloid β peptide, *Biochemistry*, 2010, **49**, 1358–1360.

5 L. Yang, K. Arora, W. A. Beard, S. H. Wilson and T. Schlick, Critical role of magnesium ions in DNA polymerase β's closing and active site assembly, *J. Am. Chem. Soc.*, 2004, **126**, 8441–8453.

6 J. S. Lee, Y. M. Bae, A. Han and S. Y. Lee, Development of Congo red broth method for the detection of biofilm-forming or slime-producing Staphylococcus sp., *LWT - Food Sci. Technol.*, 2016, **73**, 707–714.

7 D. N. De Vasconcelos and V. F. Ximenes, Albumin-induced circular dichroism in Congo red: Applications for studies of amyloid-like fibril aggregates and binding sites, *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, 2015, **150**, 321–330.

8 Y. Z. Zhang, X. Xiang, P. Mei, J. Dai, L. L. Zhang and Y. Liu, Spectroscopic studies on the interaction of Congo Red with bovine serum albumin, *Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.*, 2009, **72**, 907–914.

9 H. M. C. de Paula, Y. L. Coelho, A. J. P. Agudelo, J. de P. Rezende, G. M. D. Ferreira, G. M. D. Ferreira, A. C. dos S. Pires and L. H. M. da Silva, Kinetics and thermodynamics of bovine serum albumin interactions with Congo red dye, *Colloids Surfaces B Biointerfaces*, 2017, **159**, 737–742.

10 T. Miura, C. Yamamiya, M. Sasaki, K. Suzuki and H. Takeuchi, Binding mode of Congo Red to Alzheimer's amyloid β-peptide studied by UV Raman spectroscopy, *J. Raman Spectrosc.*, 2002, **33**, 530–535.

11 W. E. Klunk, R. F. Jacob and R. P. Mason, Quantifying amyloid by congo red spectral shift assay, *Methods Enzymol.*, 1999, **309**, 285–305.

12 B. Y. G. Scatchard, The Attractions of proteins for small molecules abd ions, *Ann. New York Acad. Sci.*, 1949, 660–672.

13 E. F. Healy, Quantitative determination of DNA-ligand binding using fluorescence spectroscopy. An undergraduate biochemistry experiment, *J. Chem. Educ.*, 2007, **84**, 1304–1307.