Article

Photodynamic Inactivation of *Legionella Pneumophila* Biofilm Formation by Cationic Tetra- and Tripyridylporphyrins in Waters of Different Hardness

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Table S1. Absorption and fluorescence properties of TMPyP3 (A), TMPyP3-CH_3 (B) and TMPyP3-$C_{17}H_{35}$ (C) in waters of different hardness (DEMI, HW and SW). Fluorescence was measured at concentration 1 μM after excitation at Soret band wavelength.

A	Absorbance properties λ_{max} (nm) (ε x 10^3 $M^{-1}cm^{-1}$)					Fluorescence propeties	
	Soret	Q_y(1-0)	Q_y (0-0)	Q_x(1-0)	Q_y(0-0)	λ_{max} (nm)	Stokes shift
DEMI	416 (247.2)	514 (14.4)	550 (2.3)	580 (3.0)	650 (0.7)	656, 708	6
HW	417 (237.7)	514 (12.6)	544 (3.9)	580 (5.0)	650 (0.7)	657, 708	7
SW	416 (250.6)	514 (13.9)	550 (3.7)	581 (5.9)	650 (0.5)	656, 707	6

B	Absorbance properties λ_{max} (nm) (ε x 10^3 $M^{-1}cm^{-1}$)					Fluorescence propeties	
	Soret	Q_y(1-0)	Q_y(0-0)	Q_x(1-0)	Q_x (0-0)	λ_{max} (nm)	Stokes shift
DEMI	418 (255.3)	516 (14.9)	553 (3.7)	581 (5.6)	648 (0.9)	655, 704	7
HW	418 (238.7)	517 (12.7)	548 (4.7)	581 (5.2)	650 (0.8)	656, 705	6
SW	418 (250.4)	516 (13.6)	553 (4.1)	581 (5.4)	650 (0.7)	656, 705	1

C	Absorbance properties λ_{max} (nm) (ε x 10^3 $M^{-1}cm^{-1}$)					Fluorescence propeties	
	Soret	Q_y(1-0)	Q_y(0-0)	Q_x(1-0)	Q_x (0-0)	λ_{max} (nm)	Stokes shift
DEMI	420 (255.0)	517 (14.7)	552 (4.0)	584 (5.5)	650 (0.8)	649, 708	/
HW	420 (159.2)	519 (12.6)	553 (5.2)	588 (4.9)	649 (1.4)	649, 708	/
SW	420 (177.4)	518 (12.5)	555 (4.5)	587 (4.0)	646 (1.4)	649, 708	3

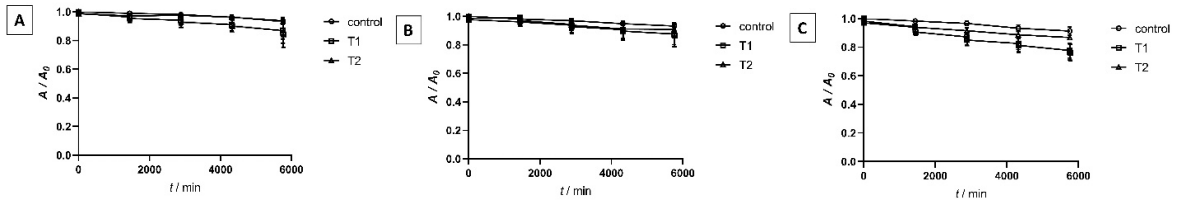

Figure S1. Photostability of TMPyP3 in DEMI (A), SW (B) and HW (C) measured by the decrease of Soret band absorption intensity in a 5-day experiment. T1 represents a 10 μM PS solution in waters of different hardness irradiated for 10 minutes every 24 h over 5 days (411 nm; total light dose 33 J/cm^2), while T2 represents samples irradiated for 10 minutes only on the first day of the experiment, and the stability was measured every 24h for 5 days (411 nm; total light dose 6.6 J/cm^2). Control represents a 10 μM solution kept in the dark for 5 days, while absorbance intensity was measured every 24 h. Data represents average of triplicate measurement with error bars presenting standard deviation.

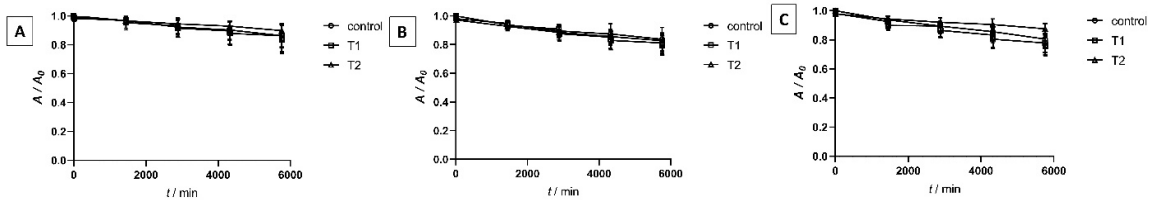

Figure S2. Photostability of TMPyP3-CH_3 in DEMI (A), SW (B) and HW (C) measured by the decrease of Soret band absorption intensity in a 5-day experiment. T1 represents a 10 μM PS solution in waters of different hardness irradiated for 10 minutes with violet light every 24 h over 5 days (411 nm; total light dose 33 J/cm^2), while T2 represents samples irradiated for 10 minutes only on the first day of the experiment, and the stability was measured every 24 h for 5 days (411 nm; total light dose 6.6 J/cm^2). Control represents a 10 μM solution kept in the dark for 5 days, while absorbance intensity was measured every 24 h. Data represents average ± SD.

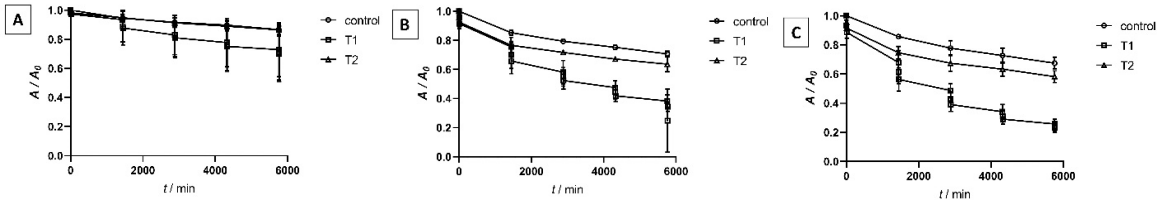

Figure S3. Photostability of TMPyP3-$C_{17}H_{35}$ in DEMI (A), SW (B) and HW (C) measured by the decrease of Soret band absorption intensity in a 5-day experiment. T1 represents a 10 μM PS solution in waters of different hardness irradiated for 10 minutes with violet light every 24 h over 5 days (411 nm; total light dose 33 J/cm^2), while T2 represents samples irradiated for 10 minutes only on the first day of the experiment, and the stability was measured every 24 h for 5 days (411 nm; total light dose 6.6 J/cm^2). Control represents a 10 μM solution kept in the dark for 5 days, while absorbance intensity was measured every 24 h. Data represents average ± SD.

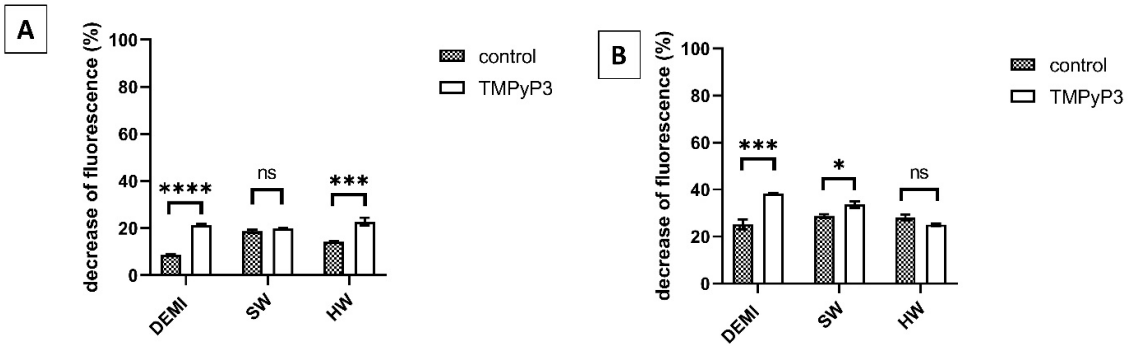

Figure S4. Singlet oxygen production of TMPyP3 (1 μM) measured by photodegradation of ABMDMA (6 μM). For irradiation, a LED-based source of violet light (411 nm) was used with a fluence rate 7 mW/cm^2 (light dose 4.2 J/cm^2) (A) and 11 mW/cm^2 (light dose 6.6 J/cm^2) (B). Control represents photodegradation of ABMDMA (6 μM) without PS. Data is presented as an average of duplicate measurement ± SD; **** $p < 0.0001$, *** $0.0001 < p < 0.001$, * $0.01 < p < 0.05$.

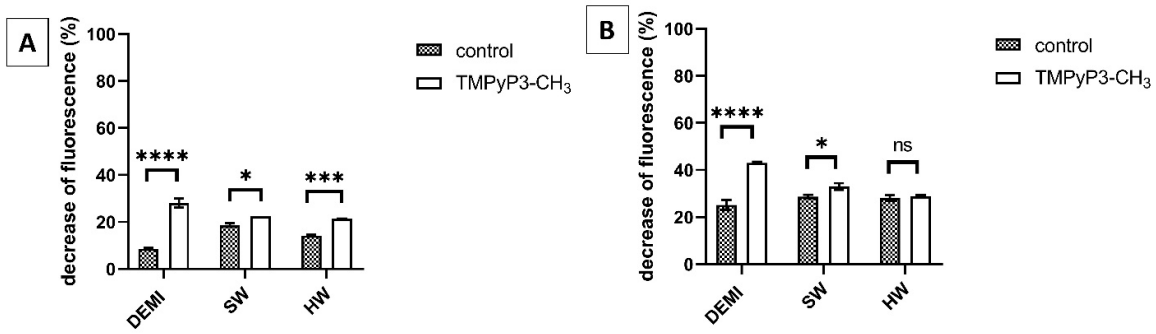

Figure S5. Singlet oxygen production of TMPyP3-CH_3 (1 μM) measured by photodegradation of ABMDMA (6 μM). For irradiation, a LED-based source of violet light (411 nm) was used with a fluence rate 7 mW/cm² (light dose 4.2 J/cm²) (A) and 11 mW/cm² (light dose 6.6 J/cm²) (B). Control represents photodegradation of ABMDMA (6 μM) without PS. Data is presented as an average of duplicate measurement ± SD; **** $p < 0.0001$, *** $0.0001 < p < 0.001$, * $0.01 < p < 0.05$.

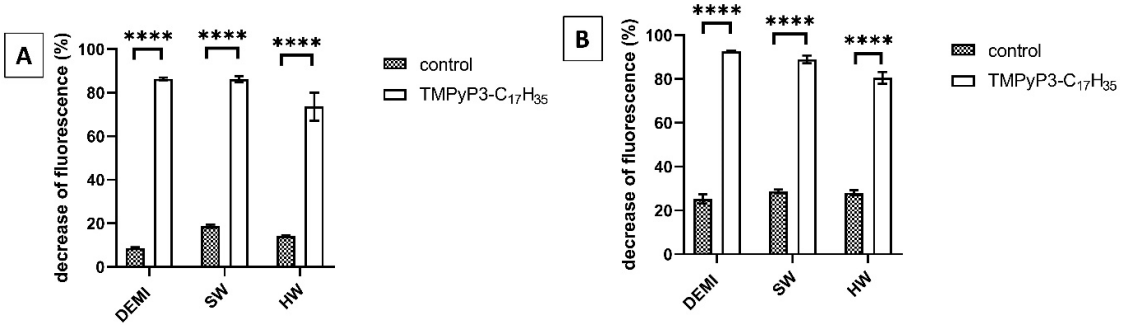

Figure S6. Singlet oxygen production of TMPyP3-$C_{17}H_{35}$ (1 μM) measured by photodegradation of ABMDMA (6 μM). For irradiation, a LED-based source of violet light (411 nm) was used with a fluence rate 7 mW/cm² (light dose 4.2 J/cm²) (A) and 11 mW/cm² (light dose 6.6 J/cm²) (B). Control represents photodegradation of ABMDMA (6 μM) without PS. Data is presented as an average of duplicate measurement ± SD; **** $p < 0.0001$.

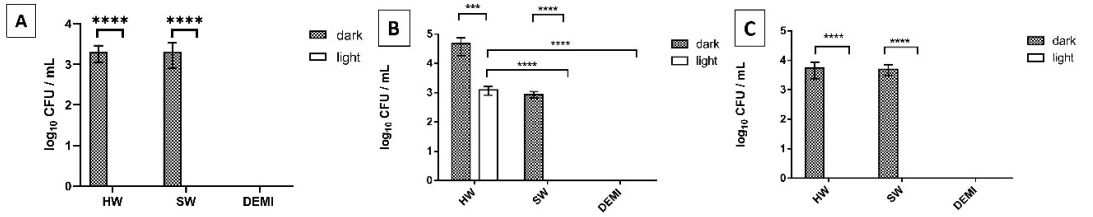

Figure S7. Impact of the PDI on the adhesion of *L. pneumophila* on polystyrene using PSs TMPyP3 (A), TMPyP3-CH_3 (B) and TMPyP3-$C_{17}H_{35}$ (C) in concentration 0.5x MEC in waters of different hardness (DEMI, SW and HW). For irradiation, a LED-based source of violet light was used for 10 min (395 nm, 20 mW/cm², 12 J/cm²). Dark control represents the samples tested with PSs without light irradiation. Data is shown as an average ± SD; .**** $p < 0.0001$, *** $0.0001 < p < 0.001$.

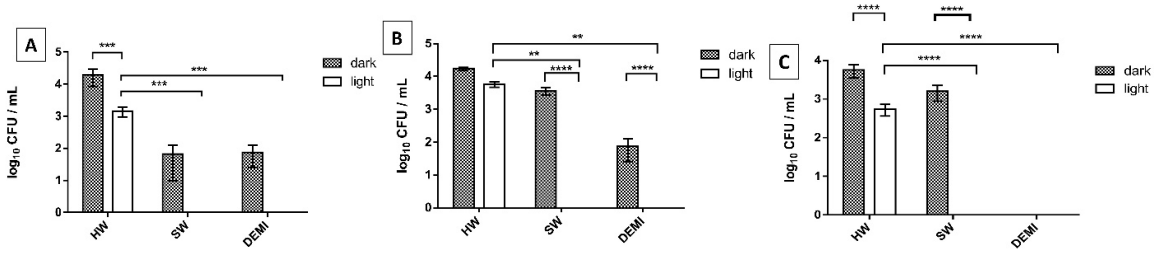

Figure S8. Impact of the PDI on the *L. pneumophila* biofilm formation using PSs TMPyP3 (A), TMPyP3-CH_3 (B) and TMPyP3-$C_{17}H_{35}$ (C) in concentration 0.5x MEC in waters of different hardness (DEMI, SW and HW). For irradiation, a LED-based source of violet light was used for 10 min (395 nm, 20 mW/cm^2, 12 J/cm^2). Dark control represents the samples tested with PSs without light irradiation. Data is shown as an average ± SD; .**** $p < 0.0001$, *** $0.0001 < p < 0.001$, ** $0.001 < p < 0.01$.

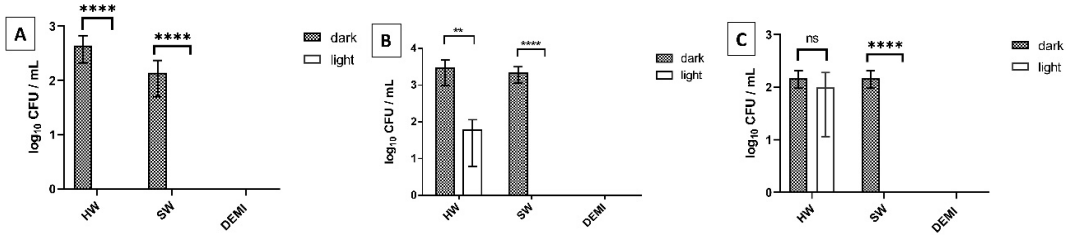

Figure S9. Impact of the PDI on the *L. pneumophila* biofilm destruction using PSs TMPyP3 (A), TMPyP3-CH_3 (B) and TMPyP3-$C_{17}H_{35}$ (C) in concentration 2x MEC in waters of different hardness (DEMI, SW and HW). For irradiation, a LED-based source of violet light was used for 10 min (395 nm, 20 mW/cm^2, 12 J/cm^2). Dark control represents the samples tested with PSs without light irradiation. Data is shown as an average ± SD; .**** $p < 0.0001$, ** $0.001 < p < 0.01$.