

Supplementary data for article:

Lippert, R.; Shubina, T. E.; Vojnovic, S.; Pavic, A.; Veselinovic, J.; Nikodinovic-Runic, J.; Stankovic, N.; Ivanović-Burmazović, I. Redox Behavior and Biological Properties of Ferrocene Bearing Porphyrins. *Journal of Inorganic Biochemistry* **2017**, *171*, 76–89.

<https://doi.org/10.1016/j.jinorgbio.2017.03.002>

Supplementary Information

for

Redox behaviour and biological properties of a novel ferrocene bearing porphyrin

Rainer Lippert¹, Tatyana E. Shubina², Sandra Vojnovic³, Aleksandar Pavic³, Jovana Veselinovic³, Jasmina Nikodinovic-Runic³, Nada Stankovic³✉, Ivana Ivanović-Burmazović¹✉

¹ Chair of Bioinorganic Chemistry, Department of Chemistry & Pharmacy, Friedrich-Alexander University Erlangen-Nuremberg, Egerlandstraße 1, D-91058 Erlangen, Germany.

² Computer Chemistry Center (CCC), Department of Chemistry & Pharmacy, Friedrich-Alexander University Erlangen-Nuremberg, Nögelsbachstraße 25, D-91052 Erlangen, Germany.

³ Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, 444a Vojvode Stepe, 11000 Belgrade, Serbia.

Table of Contents

Table S1 Bacteriostatic effect of porphyrin **4**.

Table S2 Antifungal activity of porphyrin **4**.

Table S3 Total cytotoxicity of porphyrins **1, 2, 4**, and ferrocene **3**.

Fig. S1 MIC of porphyrin **4** on *C.albicans* ATCC 10231.

Fig. S2 Effects of photoactivation on growth of *C. albicans* cultures treated with low doses of TPP **2**, ferrocene **3**, and porphyrin **4**.

Fig. S3 Effects of photoactivation on growth of *S. aureus* culture treated with low doses of TPP **2**, ferrocene **3**, and porphyrin **4**.

Fig. S4 Effects of photoactivation on growth of *B.subtilis* culture treated with low doses of TPP **2**, ferrocene **3**, and porphyrin **4**.

Fig. S5 Effects of photoactivation on growth of *K. pneumoniae* culture treated with low doses of TPP **2**, ferrocene **3**, and porphyrin **4**.

Fig. S6 Effects of photoactivation on growth of *E. faecalis* culture treated with low doses of TPP **2**, ferrocene **3**, and porphyrin **4**.

Fig. S7 Effects of photoactivation on growth of *L. monocytogenes* culture treated with low doses of TPP **2**, ferrocene **3**, and porphyrin **4**.

Fig. S8 UV/vis spectrum and fluorescence spectrum of porphyrin **1** in methylene chloride.

Fig. S9 UV/vis spectrum and fluorescence spectrum of TPP **2** in methylene chloride.

Fig. S10 Isotopic distributions of the UHR-ESI-TOF mass spectrum of porphyrin **4**.

Fig. S11 Differential pulse voltammogram of porphyrin **1** and ferrocene **3** in methylene chloride.

Fig. S12 Differential pulse voltammogram of porphyrin **4** and ferrocene **3** in methylene chloride.

Table S1 Bacteriostatic effect of sub MIC concentrations of porphyrin **4**.

Organism	MIC ($\mu\text{g/mL}$)	sub MIC ($\mu\text{g/mL}$)	CRT* (h)
<i>S. aureus</i>	31.2	15.6	12
		7.8	4
		3.9	3
<i>B. subtilis</i>	125	62.5	15
		31.2	12
		15.6	6
		7.8	4
<i>K. pneumoniae</i>	125	125	15
		62.5	5
<i>E. faecalis</i>	250	125	15
		62.5	5
		31.2	3

*CRT – culture recovery time needed for culture to enter phase of exponential growth.

Table S2 Antifungal activity of porphyrin **4** given as MIC of different pathogenic clinical fungal isolates.

Organism	MIC ($\mu\text{g/mL}$)
<i>C. albicans</i> ATCC 10231	31.2
<i>C. albicans</i> CA-06	250
<i>A. fumigatus</i> 157/10	250
<i>M. gypseum</i> 95/10	62.5

Table S3 Total cytotoxicity of porphyrins **1**, **2**, **4**, and ferrocene **3** on MRC5 human fibroblasts (IC_{50}) compared to hemolytic activity (H_{50}). Concentration range was 5-500 $\mu\text{g/mL}$ for each compound tested. IC_{50} and H_{50} are given in $\mu\text{g/mL}$.

compound	IC_{50}	H_{50}
4	20	100
1	10	300
2	500	>500
ferrocene 3	100	>500

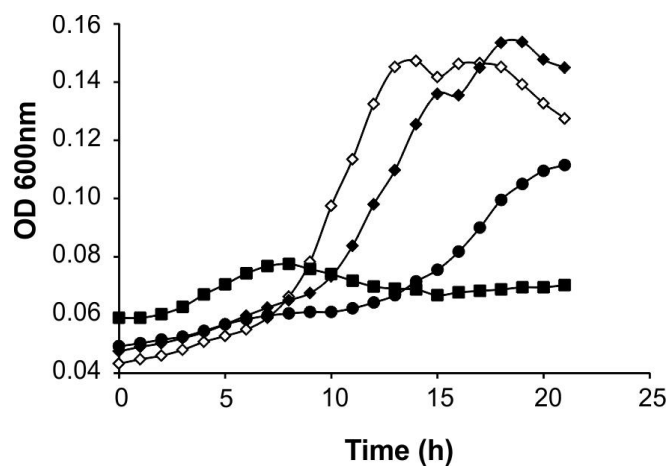


Fig. S1 MIC of porphyrin 4 on *C.albicans* ATCC 10231. \diamond Control; \blacklozenge 7.5 $\mu\text{g/mL}$;

\bullet 15.6 $\mu\text{g/mL}$; \blacksquare 31.25 $\mu\text{g/mL}$;

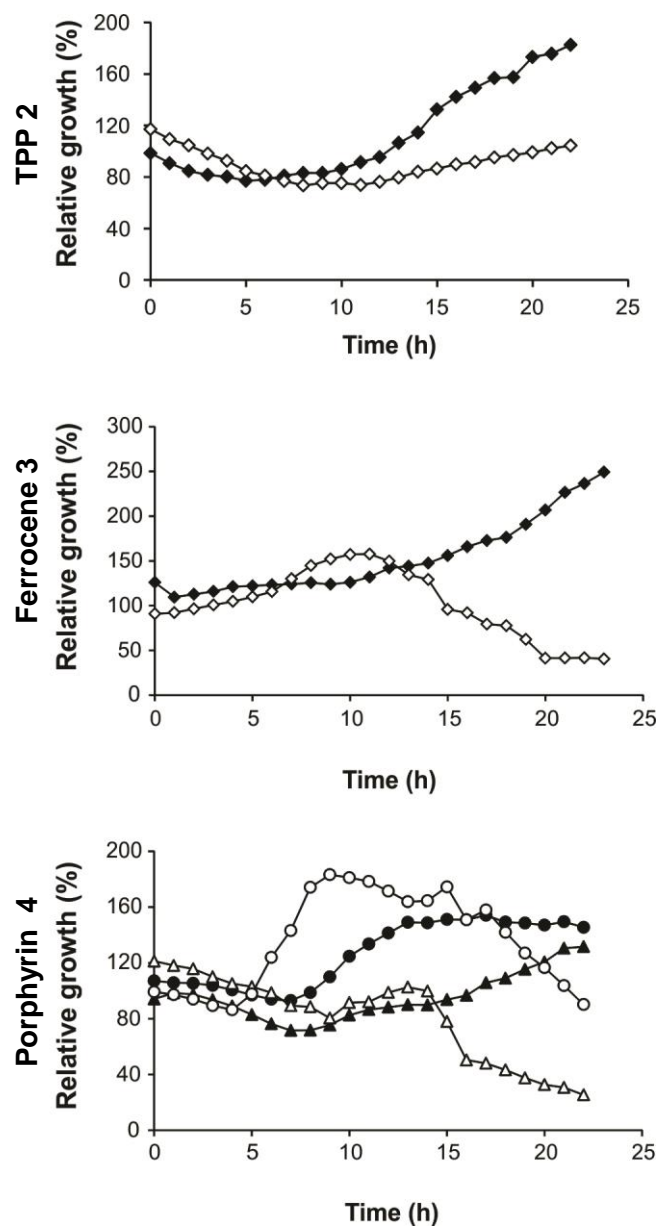


Fig. S2 Effects of photoactivation on growth of *C. albicans* cultures treated with low doses of TPP 2 (50 $\mu\text{g/mL}$), ferrocene 3 (50 $\mu\text{g/mL}$), and porphyrin 4 (7.8 $\mu\text{g/mL}$, round marks, and 15.6 $\mu\text{g/mL}$, triangular marks). Growth is given as a percent of control culture growth (100 %). Closed marks represent cultures grown in the dark, while open marks represent cultures grown upon illumination.

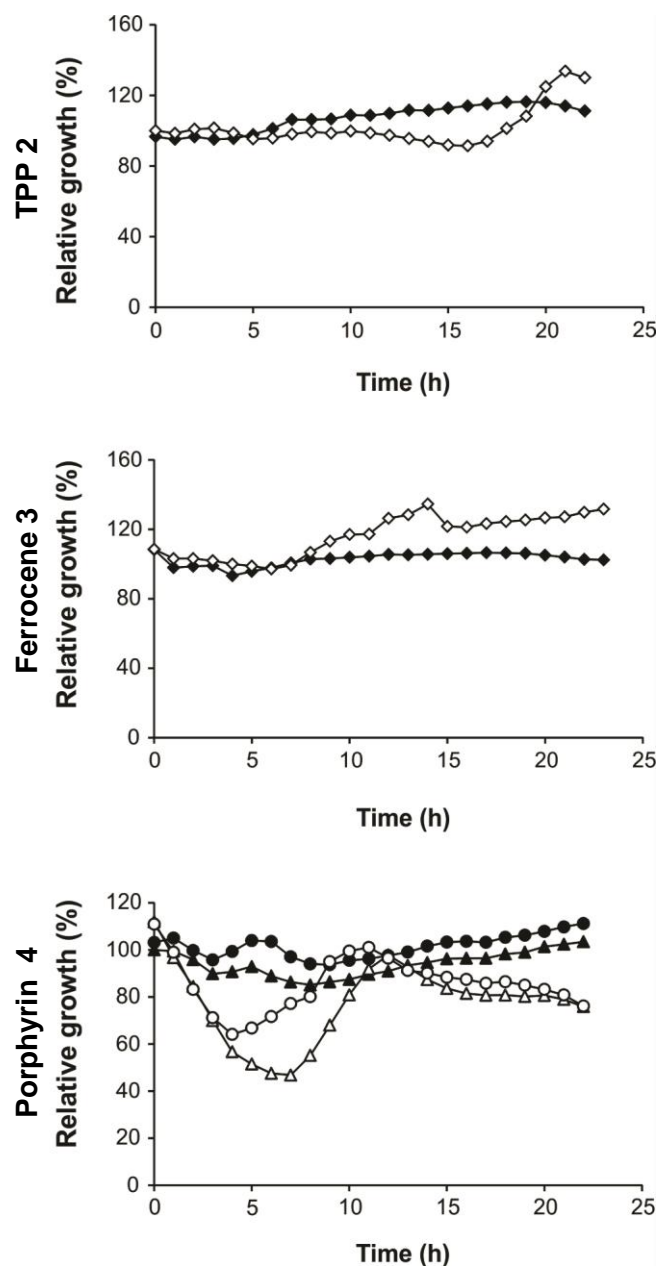


Fig. S3 Effects of photoactivation on growth of *S. aureus* culture treated with low doses of TPP **2** (50 $\mu\text{g/mL}$), ferrocene **3** (50 $\mu\text{g/mL}$), and porphyrin **4** (7.8 $\mu\text{g/mL}$, round marks, and 15.6 $\mu\text{g/mL}$, triangular marks). Growth is given as a percent of control culture growth (100 %). Closed marks represent cultures grown in the dark, while open marks represent cultures grown upon illumination.

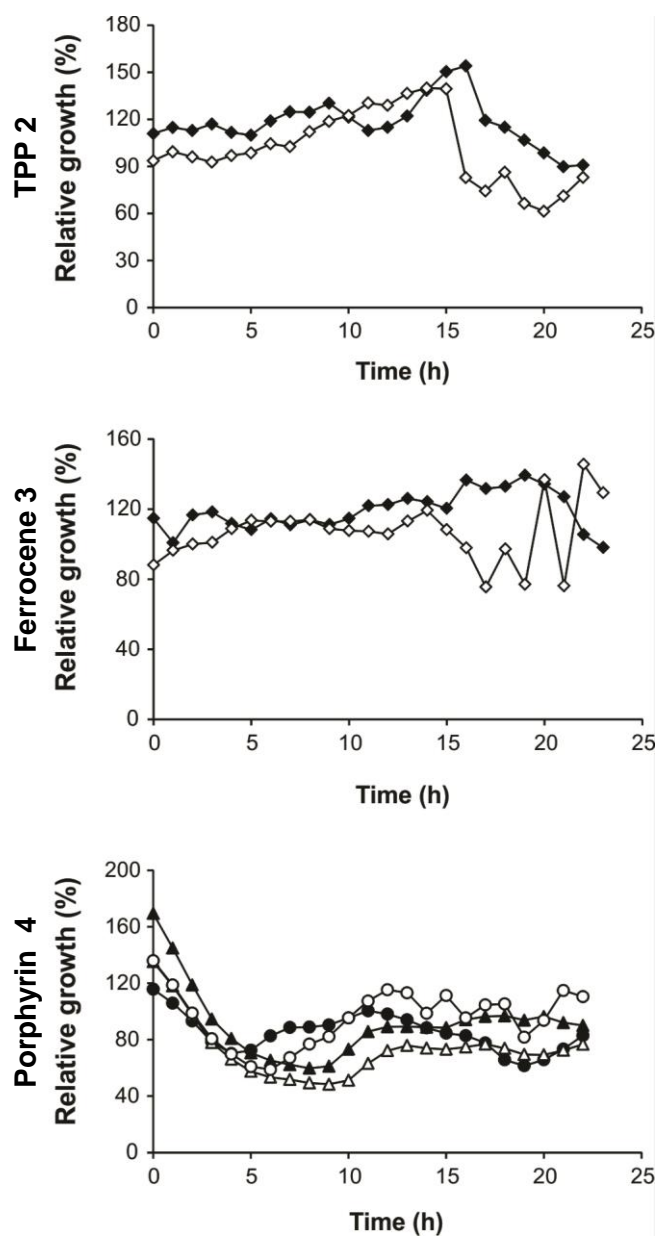


Fig. S4 Effects of photoactivation on growth of *B.subtilis* culture treated with low doses of TPP **2** (50 $\mu\text{g}/\text{mL}$), ferrocene **3** (50 $\mu\text{g}/\text{mL}$), and porphyrin **4** (31.2 $\mu\text{g}/\text{mL}$, round marks, and 62.5 $\mu\text{g}/\text{mL}$, triangular marks). Growth is given as a percent of control culture growth (100 %). Closed marks represent cultures grown in the dark, while open marks represent cultures grown upon illumination.

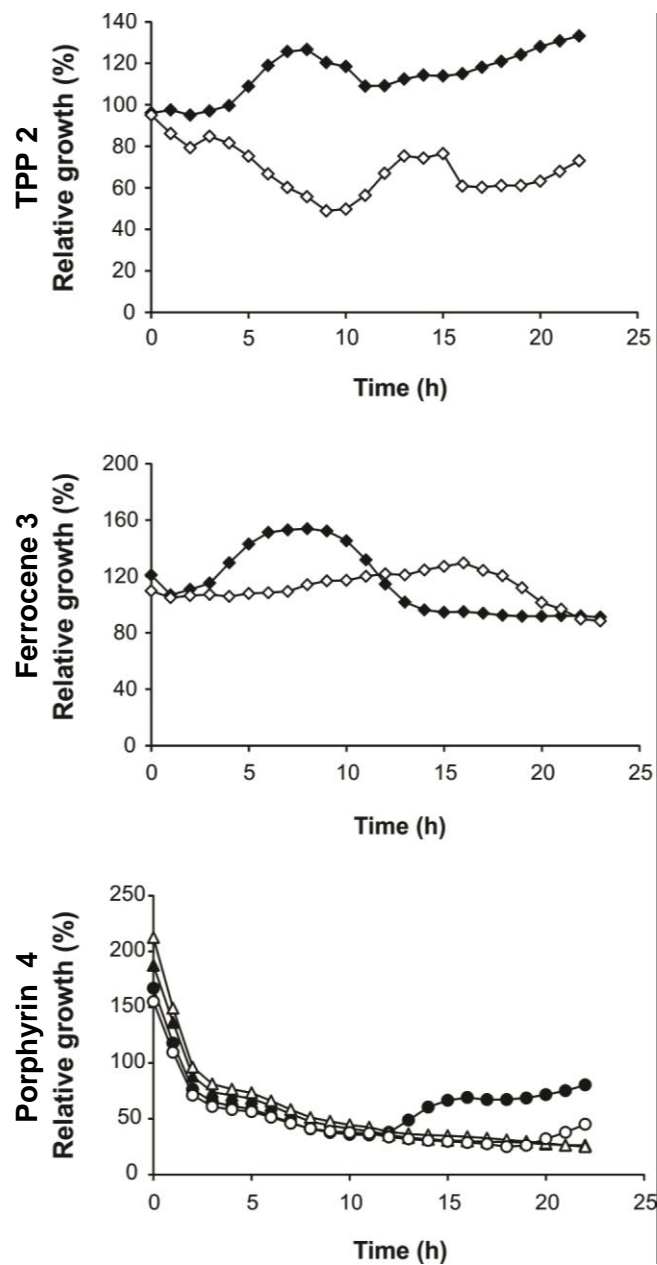


Fig. S5 Effects of photoactivation on growth of *K. pneumoniae* culture treated with low doses of TPP 2 (50 µg/mL), ferrocene 3 (50 µg/mL), and porphyrin 4 (62.5 µg/mL, round marks, and 125 µg/mL, triangular marks). Growth is given as a percent of control culture growth (100 %). Closed marks represent cultures grown in the dark, while open marks represent cultures grown upon illumination.

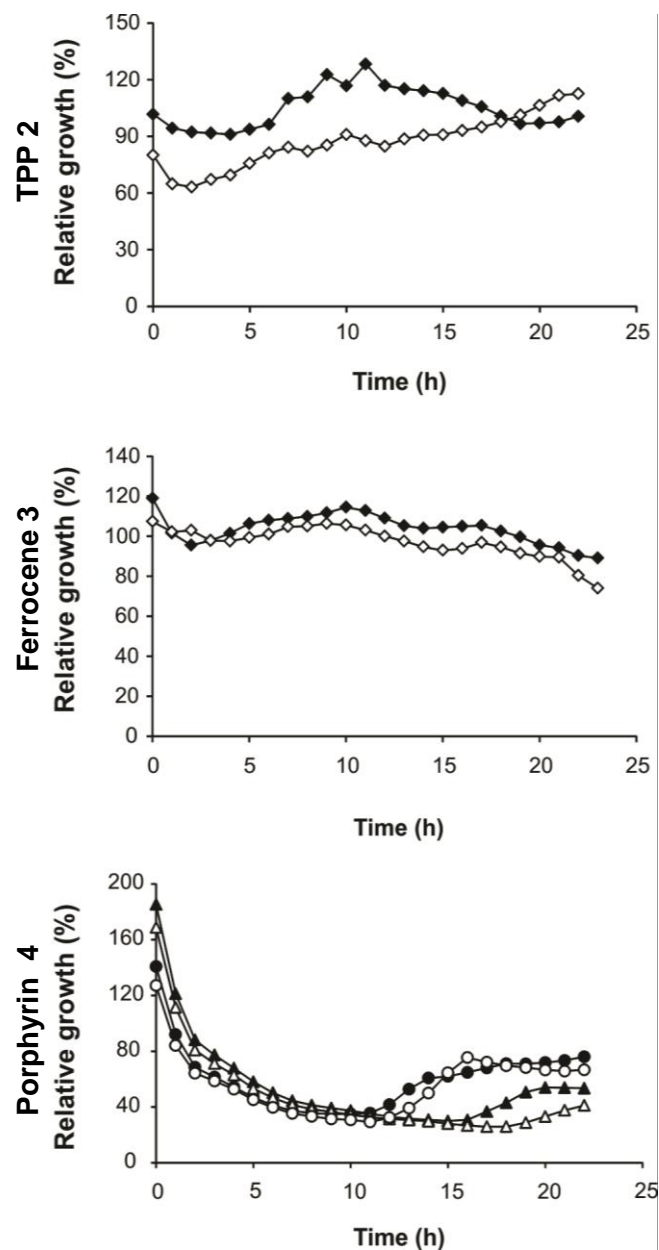


Fig. S6 Effects of photoactivation on growth of *E. faecalis* culture treated with low doses of TPP **2** (50 µg/mL), ferrocene **3** (50 µg/mL), and porphyrin **4** (62.5 µg/mL, round marks, and 125 µg/mL, triangular marks). Growth is given as a percent of control culture growth (100 %). Closed marks represent cultures grown in the dark, while open marks represent cultures grown upon illumination.

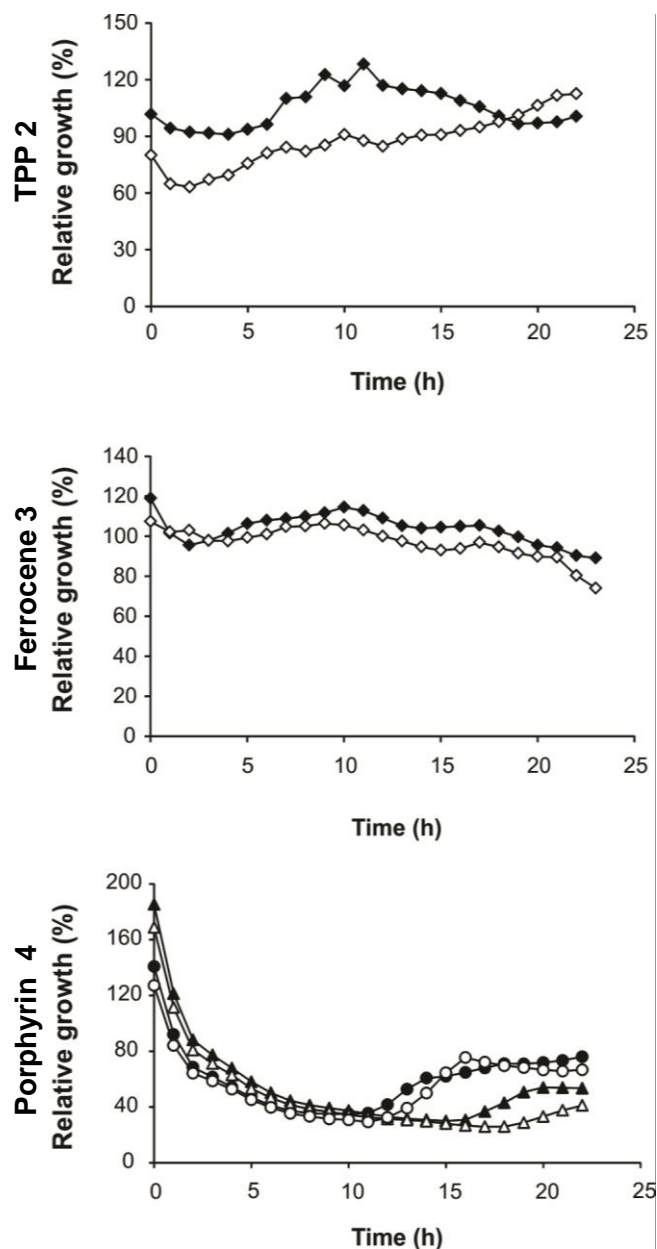


Fig. S7 Effects of photoactivation on growth of *L. monocytogenes* culture treated with low doses of ferrocene **3** (50 $\mu\text{g}/\text{mL}$), TPP **2** (50 $\mu\text{g}/\text{mL}$), and porphyrin **4** (125 $\mu\text{g}/\text{mL}$, round marks, and 250 $\mu\text{g}/\text{mL}$, triangular marks). Growth is given as a percent of control culture growth (100 %). Closed marks represent cultures grown in the dark, while open marks represent cultures grown upon illumination.

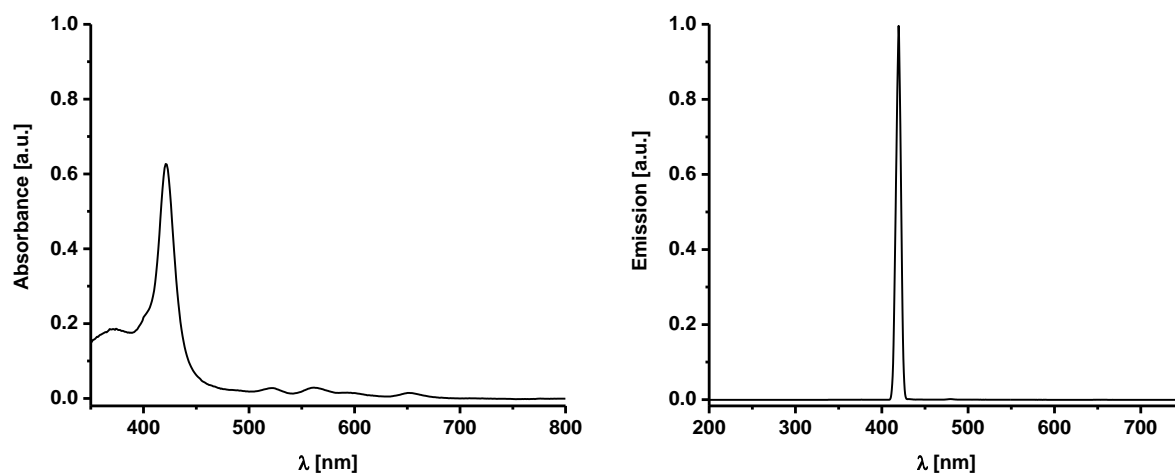


Fig. S8 UV/vis spectrum of porphyrin **1** in methylene chloride (left); Fluorescence spectrum of porphyrin **1** in methylene chloride at the excitation wavelength of 420 nm (right).

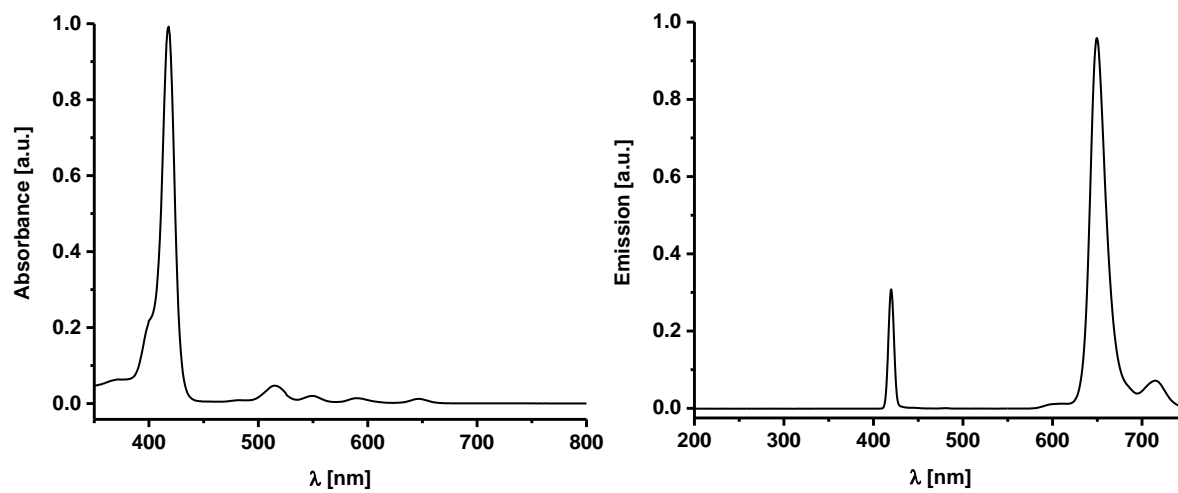
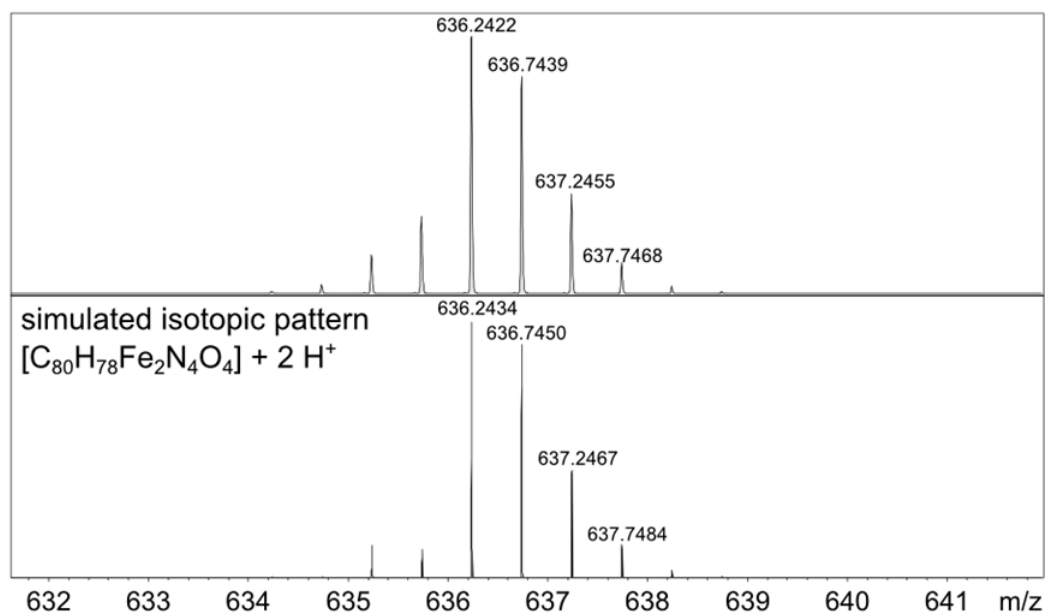


Fig. S9 UV/vis spectrum of TPP **2** in methylene chloride (left); Fluorescence spectrum of TPP **2** in methylene chloride at the excitation wavelength of 420 nm (right).

a)



b)

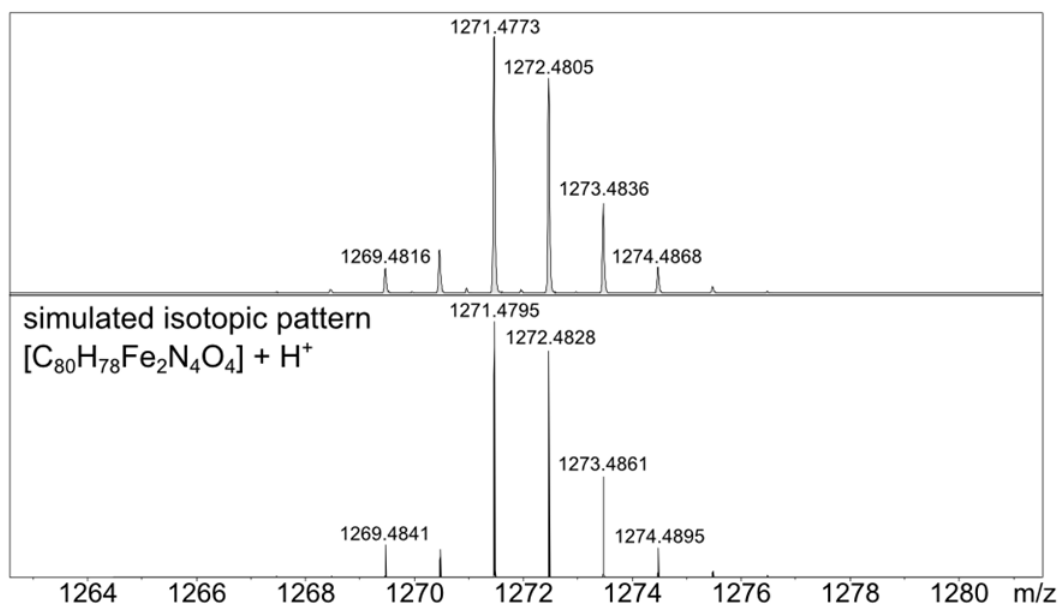


Fig. S10 Measured (top) and simulated (bottom) isotopic distributions of the peaks at m/z 636.2422 $[(C_{80}H_{78}Fe_2N_4O_4)+2H^+]$ of the UHR-ESI-TOF mass spectrum of porphyrin **4** in methylene chloride/methanol (1/1) with formic acid at RT (**a**); Measured (top) and simulated (bottom) isotopic distributions of the peaks at m/z 1271.4773 $[(C_{80}H_{78}Fe_2N_4O_4)+H^+]$ of the UHR-ESI-TOF mass spectrum of porphyrin **4** in methylene chloride/methanol (1/1) with formic acid at RT (**b**).

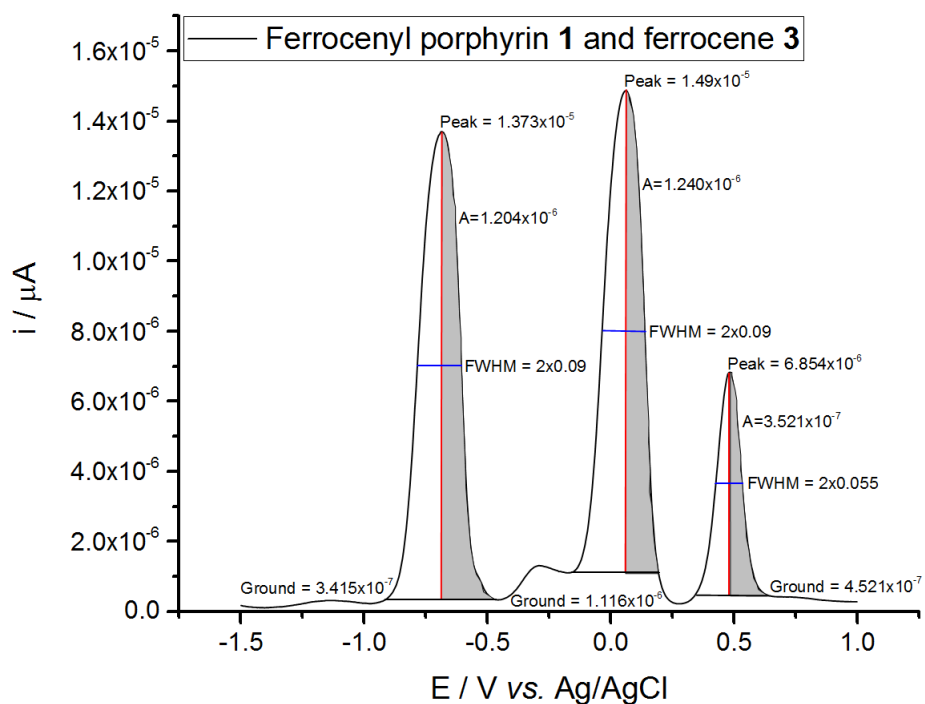


Fig. S11 Differential pulse voltammogram of a 1:1 mixture of porphyrin 1 ($c=10^{-3}$ M) and ferrocene 3 ($c=10^{-3}$ M) in methylene chloride vs. Ag/AgCl as reference.

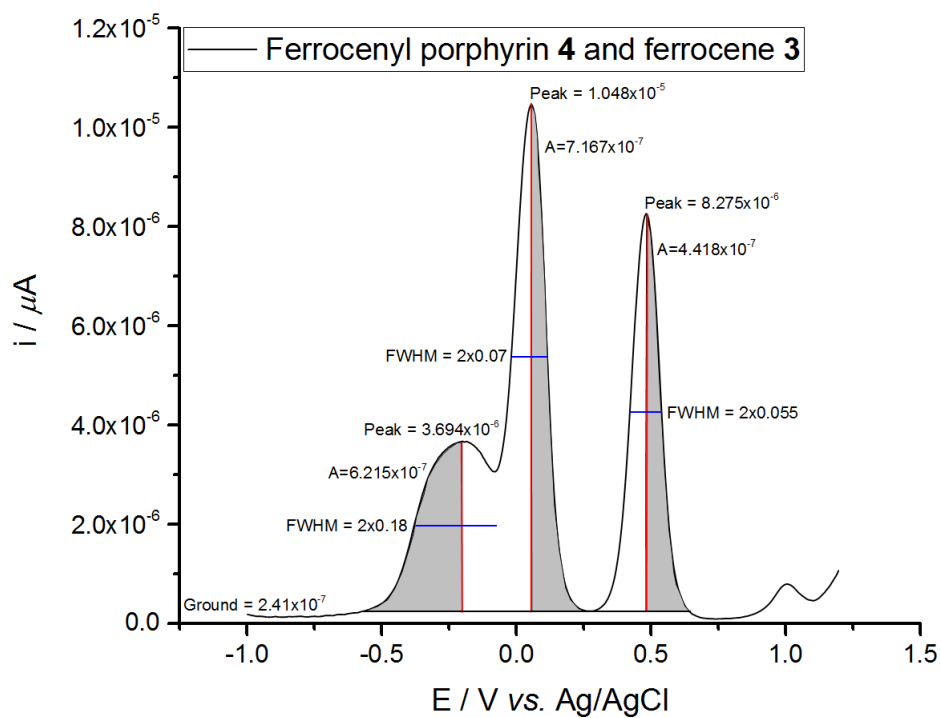


Fig. S12 Differential pulse voltammogram of a 1:1 mixture of porphyrin 4 ($c=10^{-3}$ M) and ferrocene 3 ($c=10^{-3}$ M) in methylene chloride vs. Ag/AgCl as reference.