Supporting Information

For the manuscript

Characterization of a versatile organometallic pro-drug (CORM) for experimental CO based therapeutics.

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Table S1: Equivalents of CO released to the headspace by ALF186 in distilled water (pH~5.5) in the dark, at 37°C in aerobic and anaerobic conditions

	Equivalents of CO (±0.05) released to the headspace in distilled H_2O (pH ~ 5.5), in the dark at 37°C	
Time (h)	anaerobic	aerobic
0.5	0.00	1.02
2	0.00	2.26
4	0.08	2.57
6	0.00	2.60
24	0.15	2.61

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Table S2: Equivalents of CO released to the headspace by ALF186 in several media and conditions

	Solvent					
	Saline (rt/dark/normoxic)	Saline (rt/dark/anoxic N_2)	PBS 7.4 (rt/dark/normoxic)	RPMI/FBS(10%) (rt/dark/normoxic)	RPMI/FBS(10%) (rt/dark/anoxic N_2)	FBS (rt/dark/normoxic)
Time (h)	Equivalents of CO released to the headspace					
0.5	0.77±0.03	0.05±0.04	0.93±0.03	---		---
2	2.19±0.03	0.07±0.04	2.55±0.03	1.88±0.07	0.02	2.15±0.06
4	2.34±0.04	0.09±0.04	---	2.09±0.07	0.11	2.13±0.06
6	2.24±0.04	0.00	---	2.21±0.07	0.16	2.28±0.06

Table S3: Equivalents of CO released to the headspace by ALF186 in H_2O at pH 2.5, pH~5.5, pH 8.5 and in PBS (pH 7.4), in the dark, in air, at 37°C.

Time (h)	H_2O (pH 2.5)	H_2O (pH ~ 5.5)	H_2O (pH 8.5)	PBS (pH 7.4)
2	1.3	2.3	2.7	2.7
4	1.5	2.5	2.9	2.8
6	1.4	2.5	2.7	2.8

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Table S4: Equivalents of CO released by ALF186 in PBS7.4, in air, in the dark at room temperature at 30min, 1h, 1h30min and 2h.

Time (Hours)	**PBS (pH 7.4)**
0.5	0.8
1	1.3
1.5	1.9
2	2.2

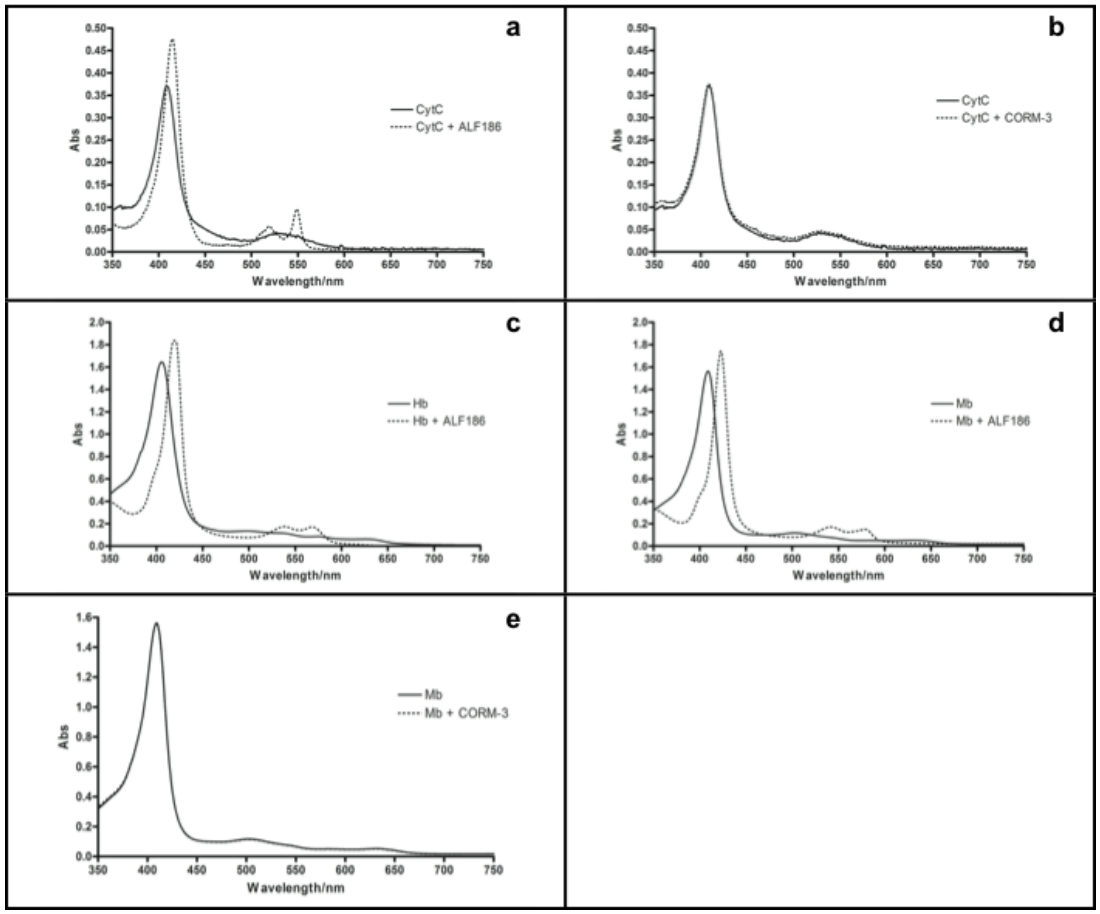

Figure S1: UV-Vis absorbance spectra of: **a)** oxidized cyt *c* (5 μM) before and immediately after incubation with ALF186 (50 μM); **b)** oxidized cyt *c* (5 μM) before and after 1 h incubation with CORM-3 (50 μM); **c**) oxidized bovine Hb (5 μM) before and immediately after incubation with ALF186 (50 μM); **d**) oxidized Mb (11 μM) before and immediately after incubation ALF186 (50 μM); **e**) oxidized Mb (11 μM) before and after 2h incubation with CORM-3 (50 μM). All spectra have been recorded in PBS7.4 at rt.

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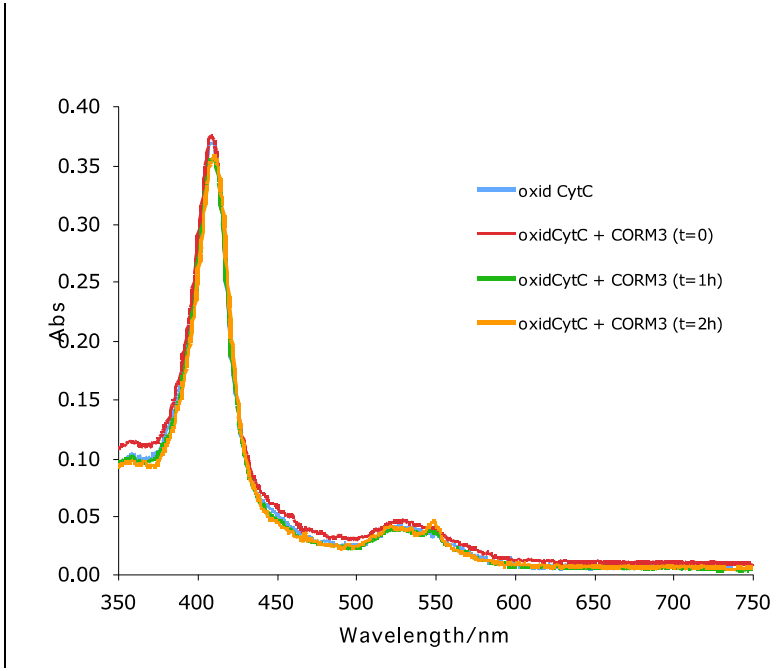

Figure S2: Time evolution of UV-Vis absorbance spectrum of oxidized cytochrome c (5 μM) before (blue line) and after addition of CORM-3 (50 μM) at t=0 (red line), 1 h (green line) and 2 h (orange line).

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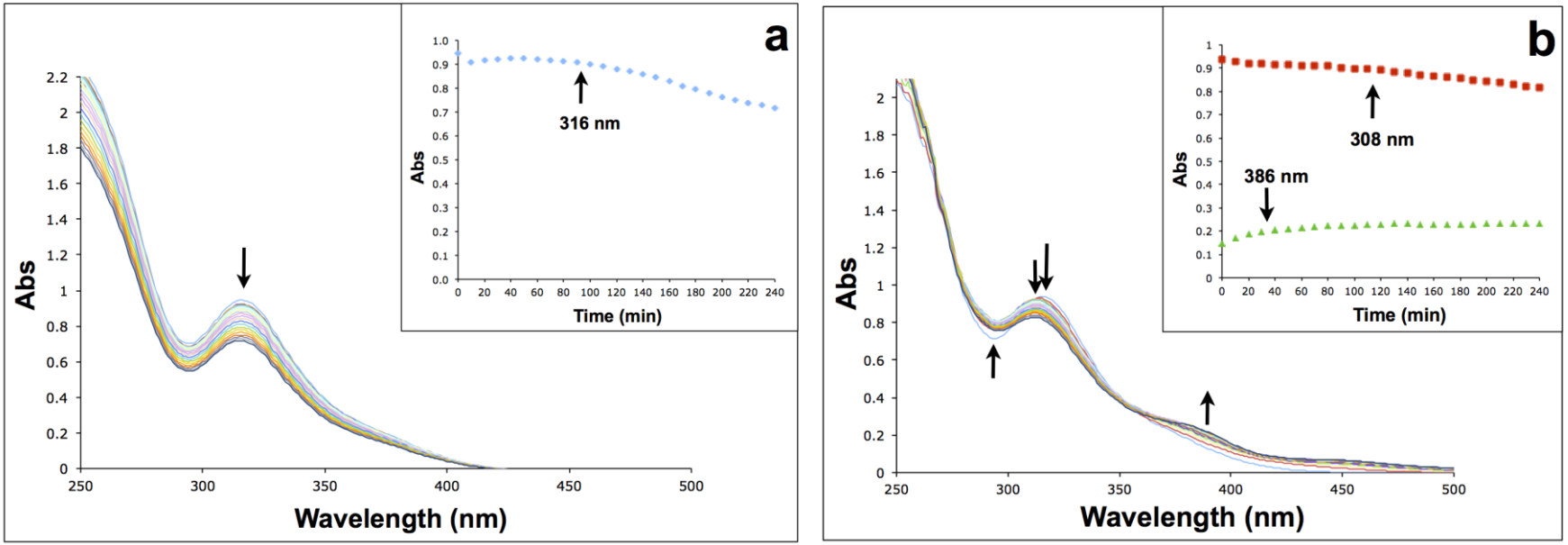

Figure S3: Time evolution of UV-Vis absorption spectrum of ALF186 in PBS7.4 at rt, under anaerobic conditions. a)pure ALF186 (250 μM); b)ALF186 and HSA (250 μM and 50 μM respectively; HSA spectrum subtracted). The variation of the absorbances with time is given in the insets.

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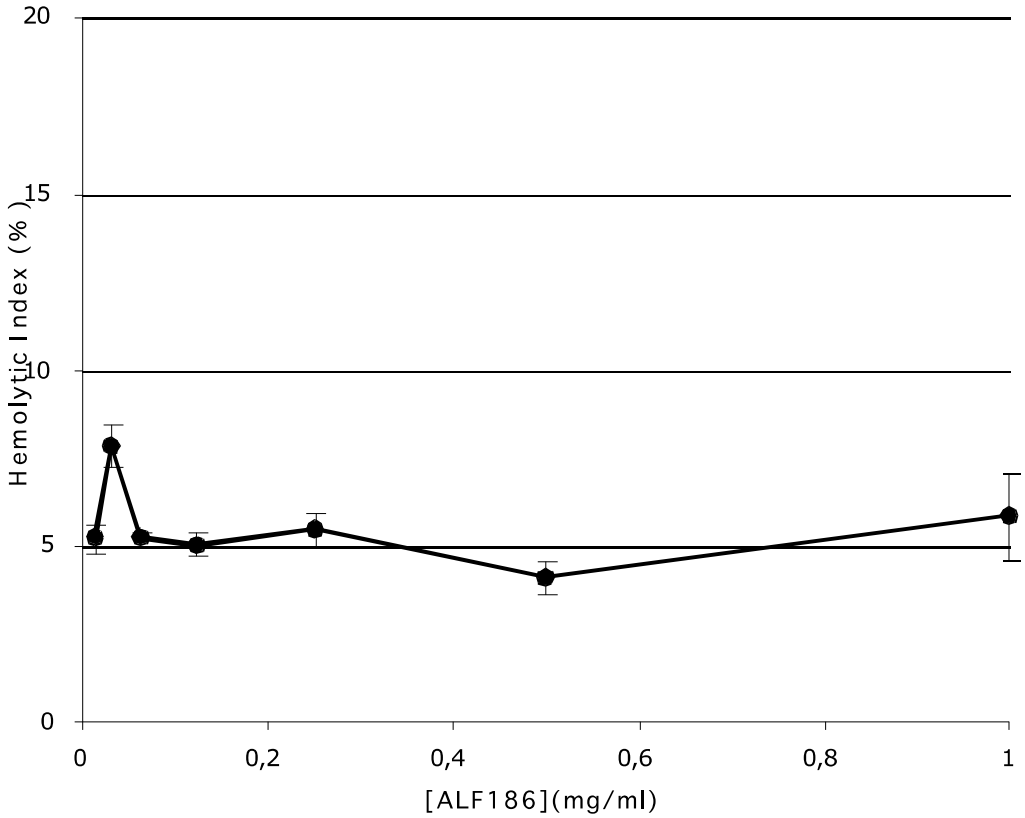

Figure S4: Hemolytic index induced by ALF186 in concentrations between 0.0078 and 1 mg/mL in sheep RBC.

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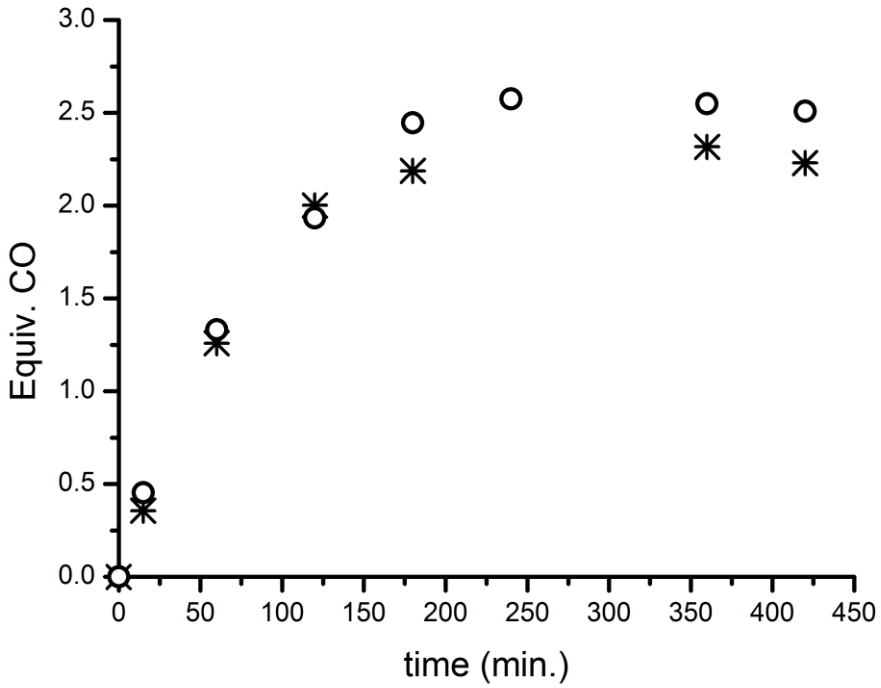

Figure S5. Equivalents of CO released from ALF186 in PBS7.4 solution (O) and in the presence of BSA, with a ratio of 41:1 (∗), along the time. An assay involving ALF186 and the BSA in an inert atmosphere at the same concentrations did not produce CO or CO_2 in the headspace.