

Supplementary Materials: Photoactivatable Surface-Functionalized Diatom Microalgae for Colorectal Cancer Targeted Delivery and Enhanced Cytotoxicity of Anticancer Complexes

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NMR Spectra

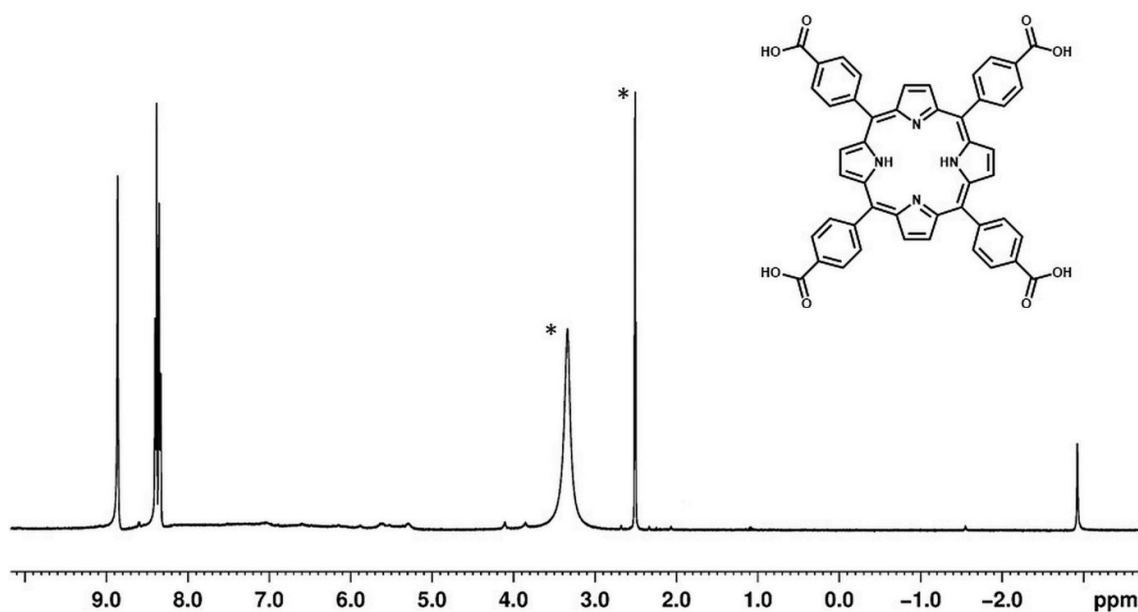


Figure S1. ¹H-NMR of TCPP. 400 MHz NMR in DMSO-d₆ (*= solvent signal).

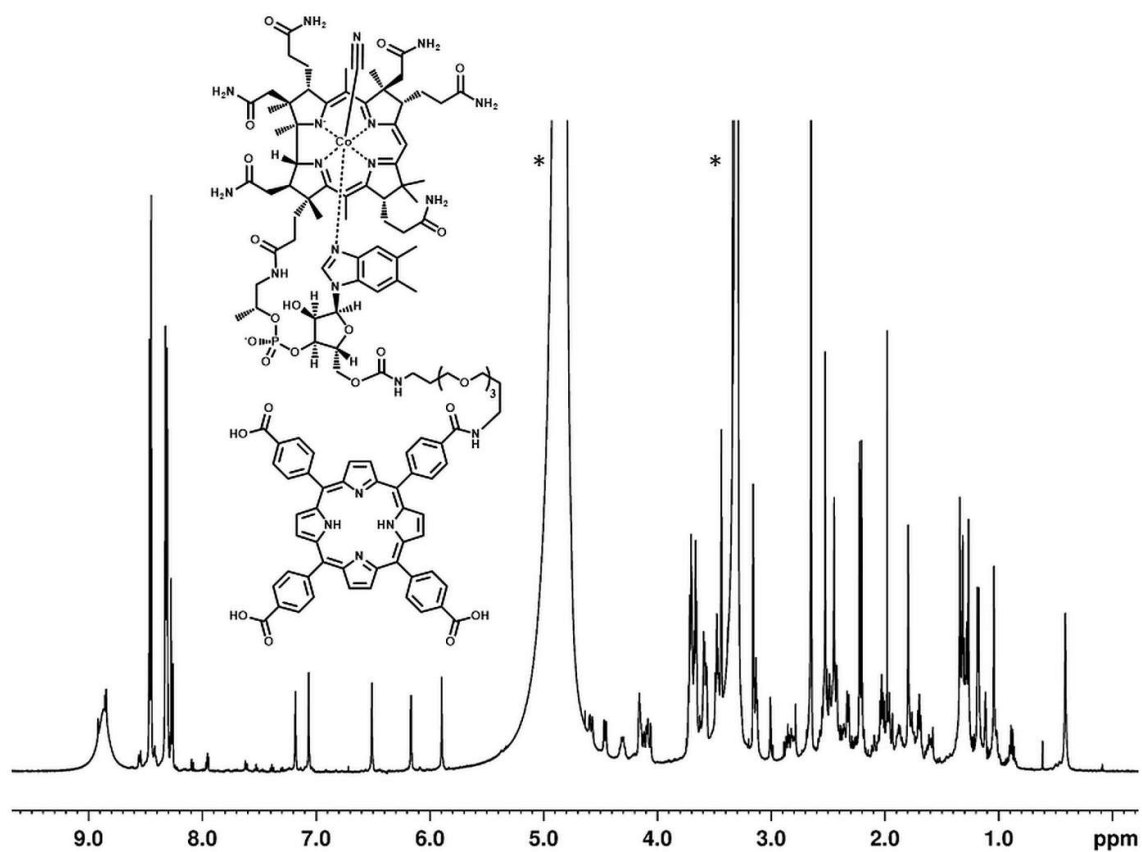


Figure S2. ^1H -NMR of $\text{B}_{12}\text{-TCPP}$. 500 MHz NMR in MeOD-d_4 (*= solvent signal).

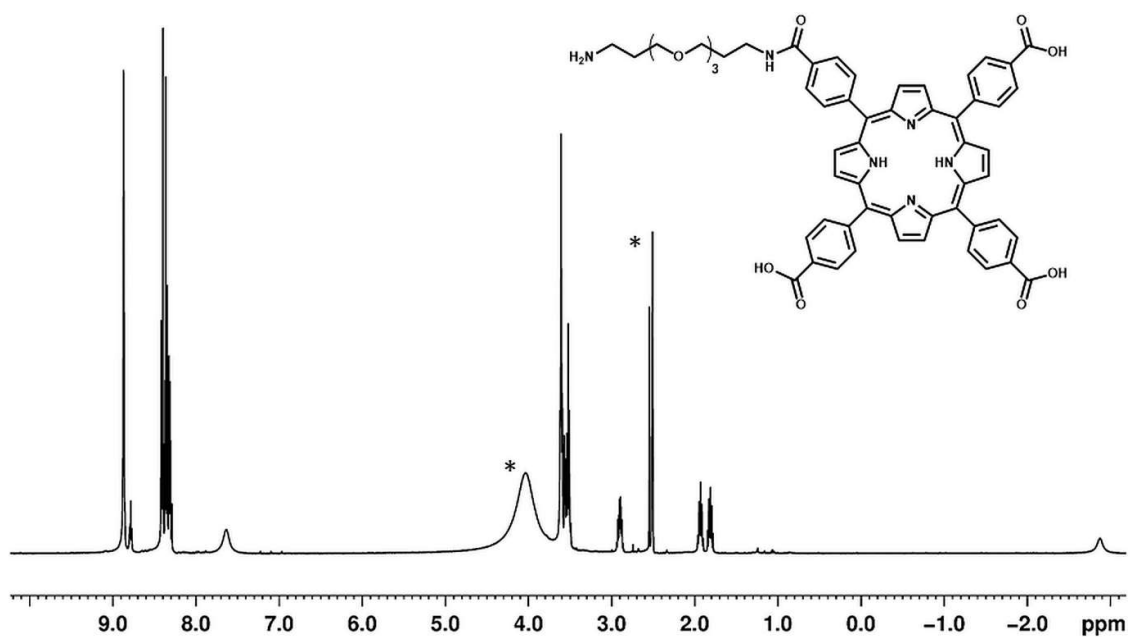


Figure S3. ^1H -NMR of TCPP-1 . 400 MHz NMR in DMSO-d_6 (*= solvent signal).

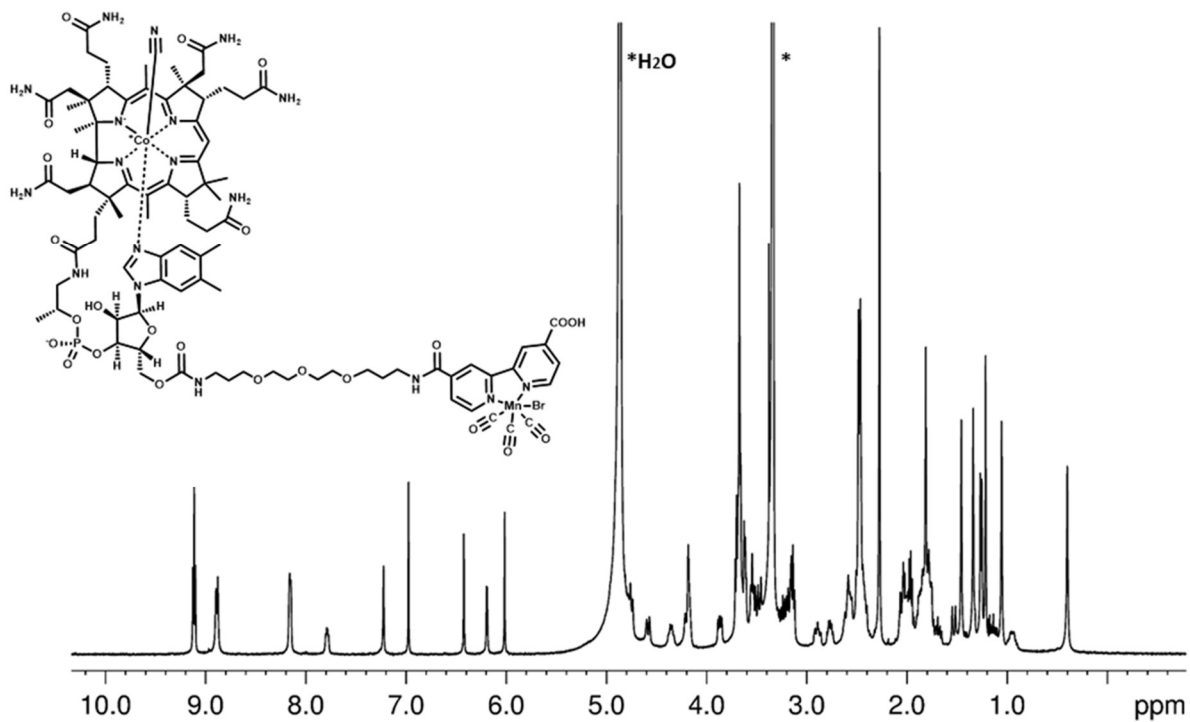


Figure S4. ^1H -NMR of $\text{B}_{12}\text{-Mn}$. 400 MHz NMR in MeOD-d_4 (*= solvent signal).

Mass Spectrometry

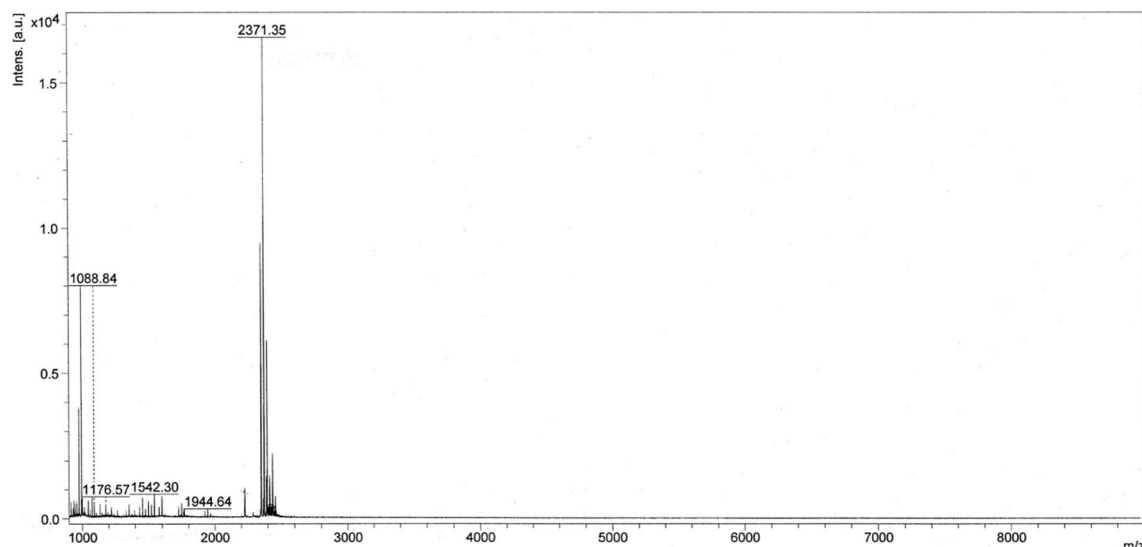


Figure S5. MS spectrum of $\text{B}_{12}\text{-TCPP}$. Measured with MALDI-TOF, 2,5-dihydroxybenzoic acid (DHB) used as matrix.

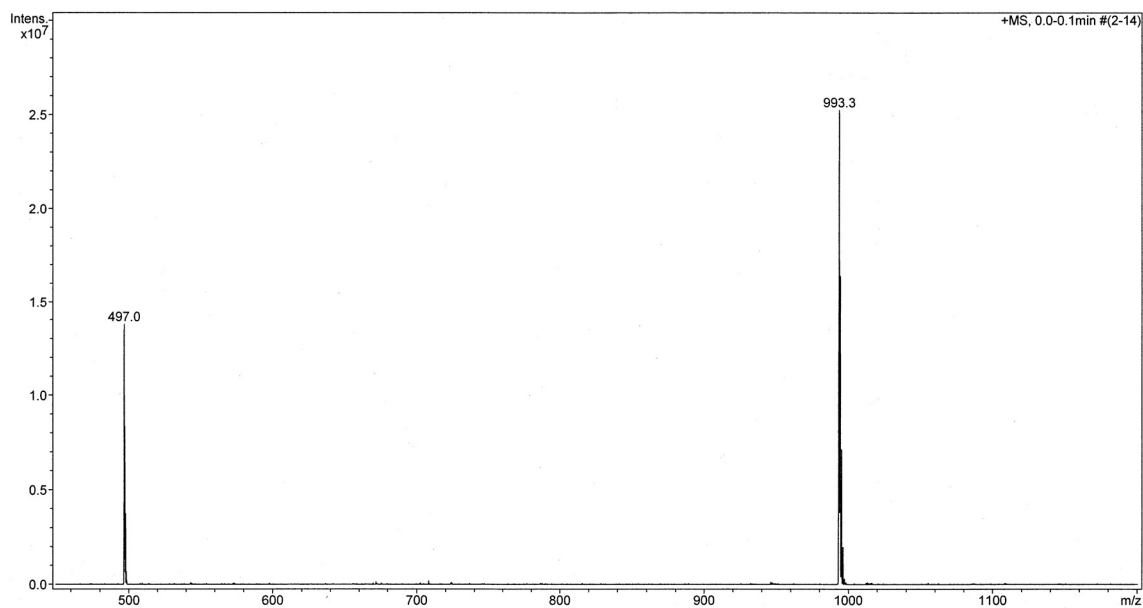


Figure S6. MS spectrum of TCPP-1. Measured with ESI-MS (positive mode) in MeOH.

Fluorescence Spectroscopy

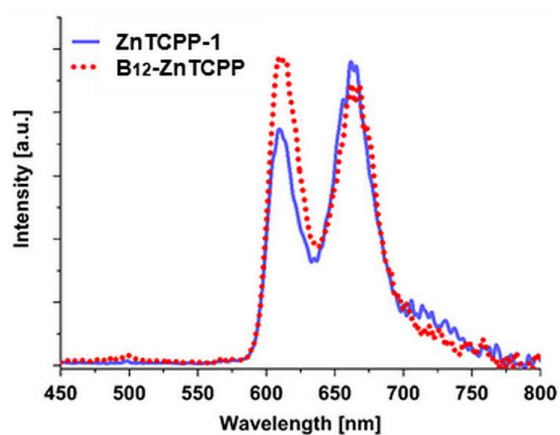


Figure S7. Fluorescence spectra of ZnTCPP-1 and B₁₂-ZnTCPP. Measured in DMSO.

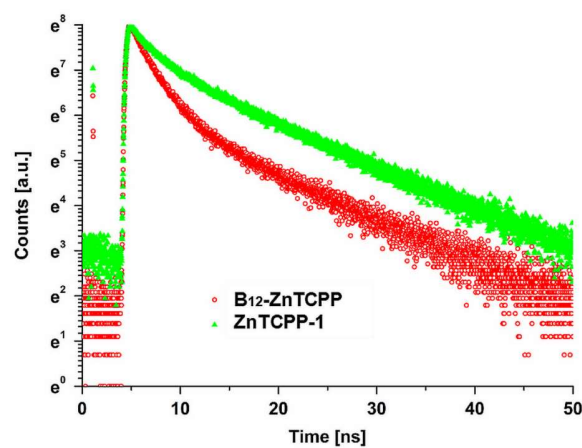


Figure S8. Fluorescence lifetime decay curves of B₁₂-ZnTCPP and ZnTCPP-1. The samples were prepared in DMSO and measurements were recorded with a TCSPC spectrophotometer.

Infrared Spectroscopy

All the IR spectra were measured with ATR system.

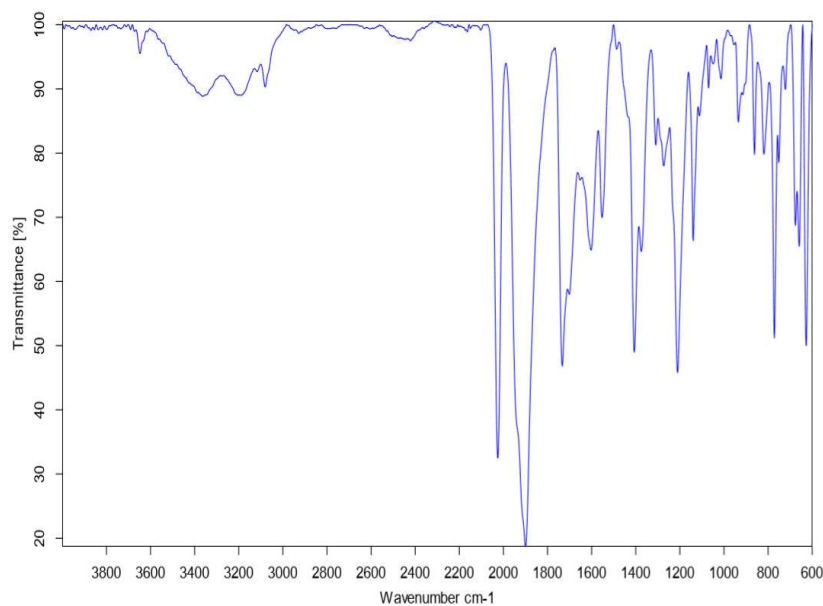


Figure S9. IR of Mn.

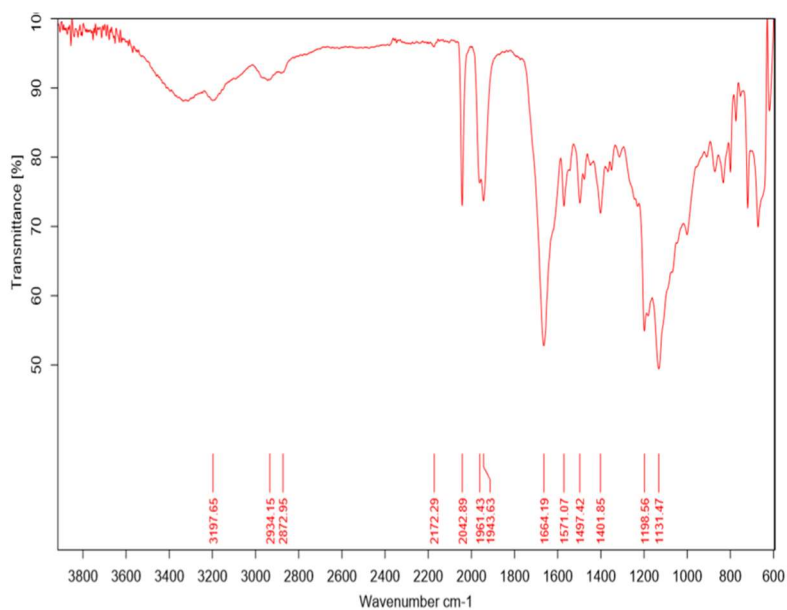


Figure S10. IR of B₁₂-Mn.

Spectra of Mn correspond to previously described analysis in literature¹.

¹ A. Ruggi and F. Zobi, "Quantum-CORMs: Quantum Dot Sensitized CO Releasing Molecules," *Dalton Trans.* 44, no. 24 (2015): 10928–31, <https://doi.org/10.1039/C5DT01681A>; Jeremie Rossier et al., "Organometallic Cobalamin Anticancer Derivatives for Targeted Prodrug Delivery via Transcobalamin-Mediated Uptake," *Dalton Trans.* 46, no. 7 (2017): 2159–64, <https://doi.org/10.1039/C6DT04443C>.

HPLC Analyse

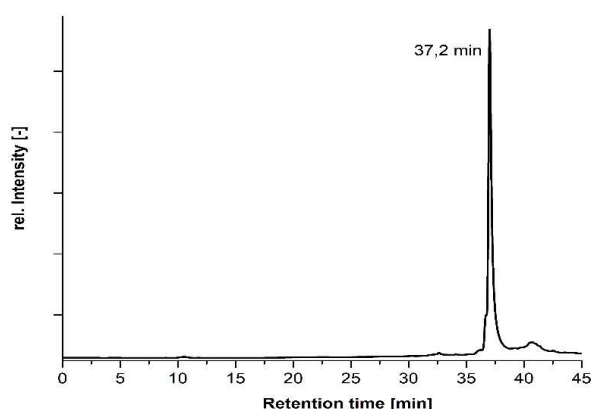


Figure S11. HPLC chromatogram of B₁₂-TCPP. For analytical HPLC a Macherey-Nagel Nucleodur C18 HTec (5 μm particle size, 110 $\text{\AA}$ pore size, 250 $\times$ 21 mm) was used. Aqueous trifluoroacetic acid 0.1% solution and pure methanol were respectively used as solvents (A) and (B). The purified compound was injected and following gradient was used: 0–5 min (50% A), 5–30 (50% A $\rightarrow$ 0% A), 30–45 min (100% B), the flow rate set to 5 mL min⁻¹ and the compound detected at 320 nm. The retention time of the compound corresponds to 37.2 minutes.

UV/Vis Spectrometry

In solution

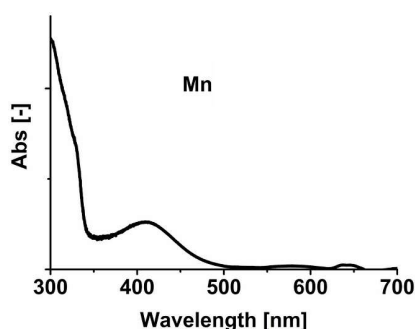


Figure S12. UV/Vis of Mn.

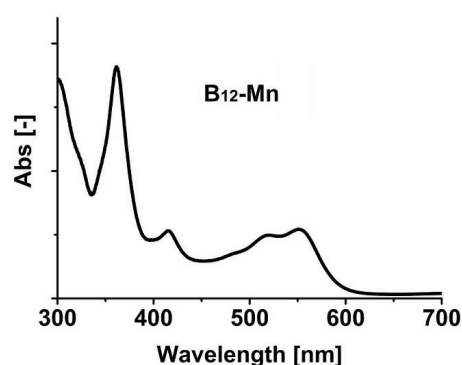


Figure S13. UV/Vis of B₁₂-Mn.

In solid-state

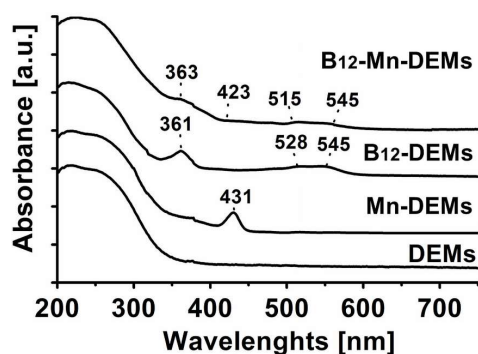


Figure S14. Solid state UV/Vis of DEMs, Mn-DEMs, B₁₂-DEMs and B₁₂-Mn-DEMs.

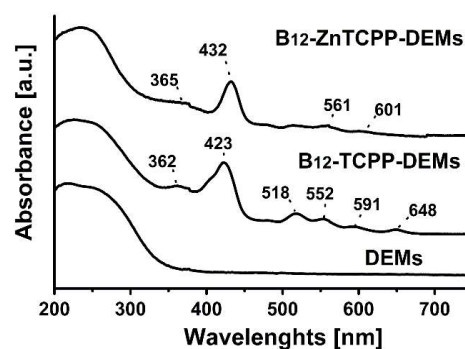


Figure S15. Solid state UV/Vis of DEMs, B₁₂-TCPP-DEMs and B₁₂-ZnTCPP-DEMs.

Equivalent of CO release

Here below are shown the concentration of Myoglobin-CO complex c(Mb-CO) formed through time with the CO release from either Mn5 (15 μM) or B₁₂-Mn5 (10 μM) upon irradiation at 420 nm under

Argon atmosphere and determined from UV/Vis spectroscopy as described by Motterlini *et al* ². The solution were measured in 0.1M PBS buffer at pH 7.4, 10 mM dithionite, with respectively 45 and 30 μ M Myoglobin for Mn and B₁₂-Mn assays. Due to its poor solubility, Mn was previously dissolved in DMSO (1% final concentration).

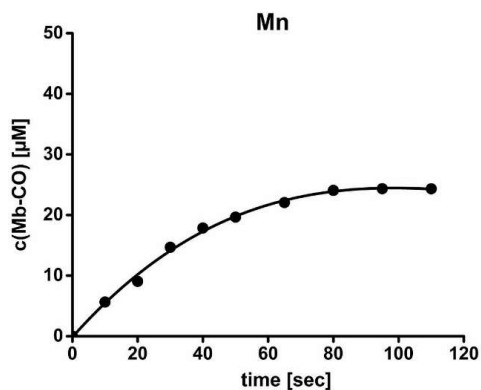


Figure S16. Concentration of Mb-CO upon light irradiation of Mn.

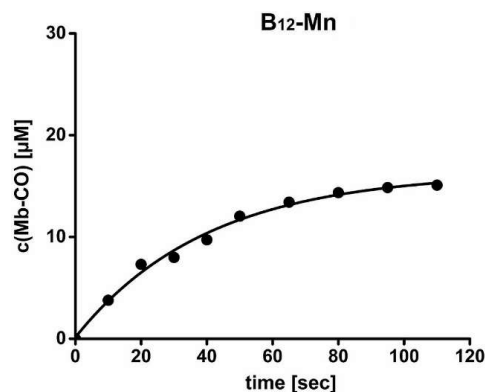


Figure S17. Concentration of Mb-CO upon light irradiation of B₁₂-Mn.

Half life

Monitoring of the spectral changes in the electronic absorption spectrum of compounds Mn and B₁₂-Mn in 0.1 M PBS upon irradiation with 420 nm light. The complexes Mn was previously dissolved in DMSO (1% final concentration).

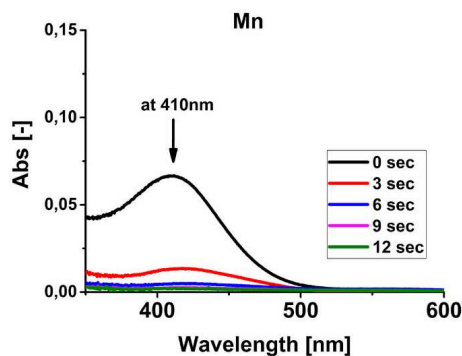


Figure S18. Spectral changes at 410 nm of Mn upon light irradiation.

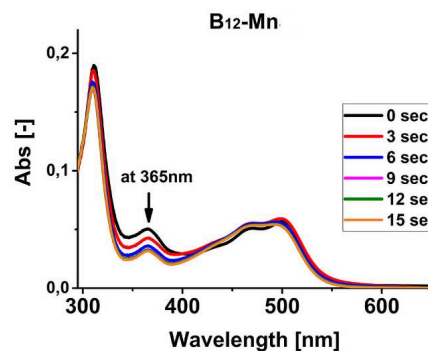


Figure S19. Spectral changes at 365 nm of B₁₂-Mn upon light irradiation.

² Roberto Motterlini and Leo E. Otterbein, "The Therapeutic Potential of Carbon Monoxide," *Nature Reviews Drug Discovery* 9 (September 1, 2010): 728.

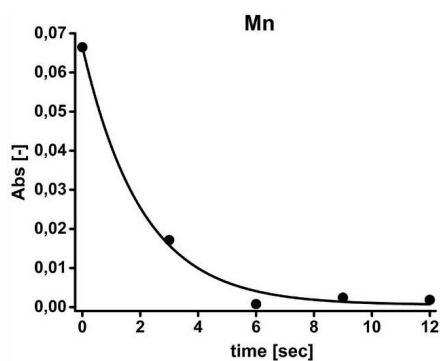


Figure S20. Mn absorption decrease at 410 nm regarding irradiation time.

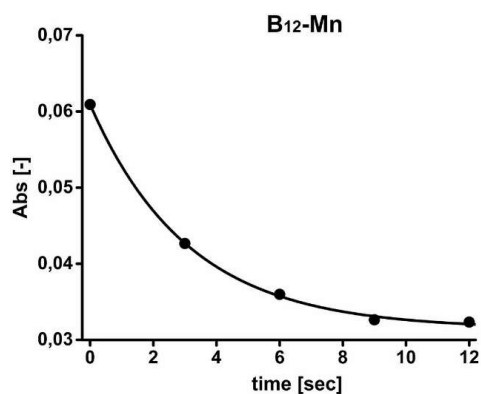


Figure S21. B₁₂-Mn absorption decrease at 365 nm regarding irradiation time.

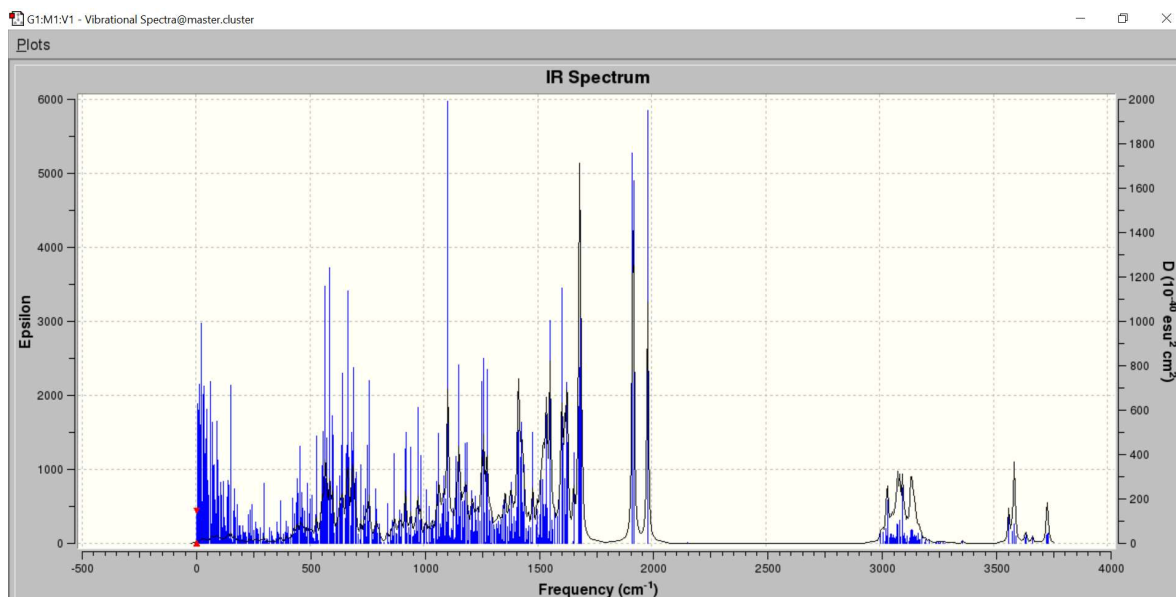


Figure S22. Calculate IR spectrum of B₁₂-Mn (gas phase b3lyp/lanl2dz).

Table S1. List of calculated IR frequencies for B₁₂-Mn (gas phase b3lyp/lanl2dz).

IR Spectrum	
X-Axis:	
Frequency	(cm ⁻¹)
Y-Axis: Epsilon	
X	Y
0.0000000000	8.8703694823
10.0000000000	27.3646439481
20.0000000000	52.1493719298
30.0000000000	75.6349061616
40.0000000000	63.8392044796
50.0000000000	71.3648041235
60.0000000000	58.5303282478

70.0000000000	99.7585892549
80.0000000000	97.4269779402
90.0000000000	112.6849065079
100.0000000000	92.9367247009
110.0000000000	68.8976208476
120.0000000000	70.0561646256
130.0000000000	64.6150408848
140.0000000000	124.0724562208
150.0000000000	126.6313021387
160.0000000000	68.7121825262
170.0000000000	81.4173881675
180.0000000000	56.8050446689

190.0000000000	47.7087173758
200.0000000000	28.0937743403
210.0000000000	29.1390839116
220.0000000000	29.3343755885
230.0000000000	57.4724829987
240.0000000000	59.6570918094
250.0000000000	38.0391230125
260.0000000000	50.9721140664
270.0000000000	50.9860892908
280.0000000000	43.8577926265
290.0000000000	46.6421515871
300.0000000000	64.5162456864
310.0000000000	25.9785862228
320.0000000000	33.3539825653
330.0000000000	39.9821438577
340.0000000000	29.6110444559
350.0000000000	38.9608016187
360.0000000000	47.8693392432
370.0000000000	88.4088372753
380.0000000000	36.3648976153
390.0000000000	54.5124317330
400.0000000000	73.5286373899
410.0000000000	55.7943127414
420.0000000000	89.0899442615
430.0000000000	206.9734909762
440.0000000000	213.6202597869
450.0000000000	195.4248546641
460.0000000000	273.4802554278
470.0000000000	225.2307725568
480.0000000000	151.7994004598
490.0000000000	204.5532306529
500.0000000000	170.8569377641
510.0000000000	190.6357080405
520.0000000000	166.7162985249
530.0000000000	327.3732258804
540.0000000000	196.6164631824
550.0000000000	509.9544471194
560.0000000000	625.3910556225
570.0000000000	973.2602225523
580.0000000000	562.2819191357
590.0000000000	666.8351022520
600.0000000000	662.6390962988
610.0000000000	233.6133759903
620.0000000000	318.8276075337
630.0000000000	560.5634858499
640.0000000000	633.6659660492

650.0000000000	396.0251763340
660.0000000000	845.5115562603
670.0000000000	790.5404516464
680.0000000000	671.4728920179
690.0000000000	985.7546629348
700.0000000000	545.7044991492
710.0000000000	190.2674211913
720.0000000000	241.2664937732
730.0000000000	177.1627364186
740.0000000000	338.6097784153
750.0000000000	505.0634366115
760.0000000000	646.0755111329
770.0000000000	182.4217308188
780.0000000000	180.3229101307
790.0000000000	308.5410493567
800.0000000000	195.0124799580
810.0000000000	82.9097330300
820.0000000000	40.8160232582
830.0000000000	51.4044796002
840.0000000000	163.5839087296
850.0000000000	117.0005979515
860.0000000000	220.1677221017
870.0000000000	375.2079527671
880.0000000000	187.1167977115
890.0000000000	274.1323876439
900.0000000000	301.1053026104
910.0000000000	225.7512583921
920.0000000000	709.7666583025
930.0000000000	235.0653593459
940.0000000000	340.4706779580
950.0000000000	213.9830261959
960.0000000000	254.4931127636
970.0000000000	498.5347948044
980.0000000000	390.8255857804
990.0000000000	233.4665012232
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2150.0000000000	1.5532344019
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3440.0000000000	1.5085691864
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3460.0000000000	1.9591045076
3470.0000000000	2.2999964427
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3500.0000000000	4.4040593135
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3530.0000000000	11.3799272190
3540.0000000000	18.4464979817
3550.0000000000	37.0388654448
3560.0000000000	132.9114123435
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3580.0000000000	326.0118688193
3590.0000000000	1044.8748415546
3600.0000000000	287.7505826696
3610.0000000000	68.5531883668
3620.0000000000	36.0641527324
3630.0000000000	40.8019631205
3640.0000000000	142.0984319597
3650.0000000000	50.8718155315
3660.0000000000	27.0089001432
3670.0000000000	99.8981814843
3680.0000000000	25.8203782414
3690.0000000000	13.4320401908
3700.0000000000	13.4419526585
3710.0000000000	19.7100788901
3720.0000000000	44.5893333203
3730.0000000000	237.0520080876
3740.0000000000	388.6444388066
3750.0000000000	59.2744713958
3760.0000000000	20.0530020174
3770.0000000000	10.0901673110
3780.0000000000	6.0960045482
3790.0000000000	4.0969656680
3800.0000000000	2.9058685037
3810.0000000000	2.1275934339
3820.0000000000	1.6579069363
3830.0000000000	1.3289712633
3840.0000000000	1.0343134868
3850.0000000000	0.8605382923
3860.0000000000	0.7271424908

3870.0000000000	0.6225201484
3880.0000000000	0.5389540704
3890.0000000000	0.4711509119
3900.0000000000	0.0000000000
3910.0000000000	0.0000000000
3920.0000000000	0.0000000000
3930.0000000000	0.0000000000

3940.0000000000	0.0000000000
3950.0000000000	0.0000000000
3960.0000000000	0.0000000000
3970.0000000000	0.0000000000
3980.0000000000	0.0000000000
3990.0000000000	0.0000000000

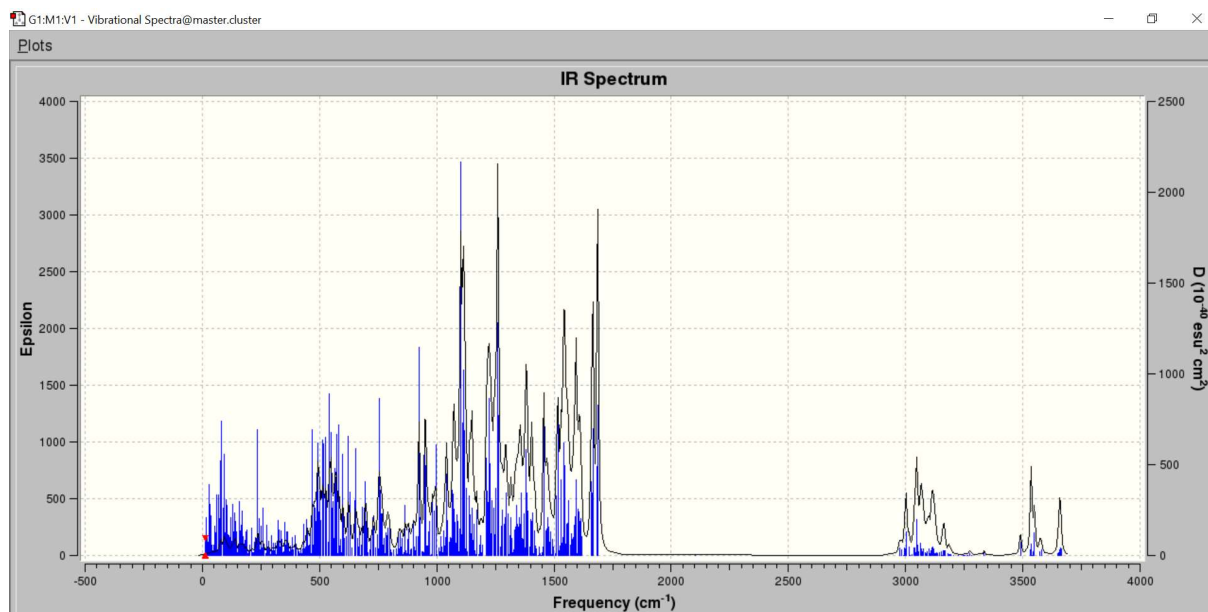


Figure S23. Calculate IR spectrum of B₁₂-TCPP (gas phase b3lyp/lanl2dz).

Table S2. List of calculated IR frequencies for B₁₂-TCPP (gas phase b3lyp/lanl2dz).

IR Spectrum			
X-Axis: Frequency (cm ⁻¹)			
Y-Axis: Epsilon			
X	Y		
0.0000000000	2.6142602577	230.0000000000	99.4033919341
10.0000000000	5.8185099518	240.0000000000	171.0795681743
20.0000000000	14.2430170126	250.0000000000	115.5962626078
30.0000000000	26.5527897094	260.0000000000	103.5121174834
40.0000000000	23.1090105894	270.0000000000	46.5121498780
50.0000000000	27.5800716911	280.0000000000	73.9778934802
60.0000000000	46.4354538431	290.0000000000	44.9320957279
70.0000000000	59.9278385877	300.0000000000	64.7727275062
80.0000000000	153.9651727780	310.0000000000	61.7518339405
90.0000000000	128.5619690614	320.0000000000	117.3350900596
100.0000000000	129.1216152766	330.0000000000	127.5254172964
110.0000000000	79.2122268816	340.0000000000	80.3875584662
120.0000000000	72.4322755149	350.0000000000	112.1234154303
130.0000000000	95.6137853143	360.0000000000	129.5237127389
140.0000000000	149.4077435430	370.0000000000	77.2626559944
150.0000000000	69.3283884068	380.0000000000	69.9329683535
160.0000000000	92.9083030282	390.0000000000	65.2524224097
170.0000000000	109.6162394514	400.0000000000	86.8954873260
180.0000000000	68.5103089316	410.0000000000	51.6701385330
190.0000000000	58.6819536876	420.0000000000	56.1533984661
200.0000000000	40.0923993356	430.0000000000	120.4544936096
210.0000000000	61.7600937621	440.0000000000	139.4064358181
220.0000000000	53.1503854407	450.0000000000	241.1316928174
		460.0000000000	207.5240066861
		470.0000000000	472.7097748270
		480.0000000000	471.8882790843
		490.0000000000	692.7605171353

500.0000000000	702.9368158056
510.0000000000	552.5967375924
520.0000000000	493.7459451901
530.0000000000	532.5361820288
540.0000000000	697.9585142109
550.0000000000	825.4933755674
560.0000000000	529.5392735214
570.0000000000	724.2714592830
580.0000000000	488.9166771483
590.0000000000	315.1851667836
600.0000000000	433.5869357057
610.0000000000	189.1395709832
620.0000000000	367.3814529139
630.0000000000	341.8795990959
640.0000000000	144.4738803960
650.0000000000	318.6193778819
660.0000000000	306.8649582005
670.0000000000	238.6169293083
680.0000000000	278.4678157403
690.0000000000	287.3310665206
700.0000000000	426.9506017191
710.0000000000	130.5863659894
720.0000000000	177.0524656803
730.0000000000	354.2063959549
740.0000000000	208.0288381716
750.0000000000	547.3435252677
760.0000000000	617.9510740567
770.0000000000	348.5904170704
780.0000000000	236.0247490227
790.0000000000	366.0906562335
800.0000000000	285.6965010068
810.0000000000	89.0766131899
820.0000000000	63.2689928655
830.0000000000	110.9429831785
840.0000000000	235.4663353101
850.0000000000	218.3354270571
860.0000000000	175.9711104497
870.0000000000	223.5356540350
880.0000000000	281.6548129792
890.0000000000	201.8857491374
900.0000000000	306.1596787713
910.0000000000	270.7368603212
920.0000000000	736.6679630594
930.0000000000	500.1623704383
940.0000000000	313.2159084469
950.0000000000	1316.2797547522

960.0000000000	519.4986528079
970.0000000000	401.9629560312
980.0000000000	510.1918385849
990.0000000000	530.9565351924
1000.0000000000	358.7034395754
1010.0000000000	184.9342548066
1020.0000000000	274.8754480105
1030.0000000000	440.3591671507
1040.0000000000	998.1808956442
1050.0000000000	429.2081918206
1060.0000000000	454.5937119475
1070.0000000000	1209.8121582573
1080.0000000000	933.7847894852
1090.0000000000	866.2844334261
1100.0000000000	2540.7599417810
1110.0000000000	2486.2955386606
1120.0000000000	1932.0279856256
1130.0000000000	731.3195723856
1140.0000000000	1098.9405175303
1150.0000000000	1310.1107544356
1160.0000000000	599.2818513824
1170.0000000000	570.9575158560
1180.0000000000	276.2907063692
1190.0000000000	336.2035833266
1200.0000000000	305.1608381750
1210.0000000000	1181.6827102515
1220.0000000000	1845.0597599949
1230.0000000000	1517.8835123284
1240.0000000000	1130.9917445061
1250.0000000000	1632.3626367296
1260.0000000000	3512.7443090660
1270.0000000000	888.5940053427
1280.0000000000	795.7994560391
1290.0000000000	827.9036433715
1300.0000000000	698.1037387716
1310.0000000000	466.5025555234
1320.0000000000	571.7366376608
1330.0000000000	618.3042955483
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1360.0000000000	995.8111476653
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1380.0000000000	1627.4329728115
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1400.0000000000	840.8098052614
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1440.0000000000	226.3196020397
1450.0000000000	523.0775759043
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1470.0000000000	856.5524854544
1480.0000000000	559.7019457682
1490.0000000000	363.8007896753
1500.0000000000	345.2527631234
1510.0000000000	898.6173651667
1520.0000000000	1103.1565348518
1530.0000000000	1281.3929037367
1540.0000000000	1994.1379350543
1550.0000000000	1574.3870662322
1560.0000000000	1264.6254309025
1570.0000000000	754.5592948699
1580.0000000000	833.5714195506
1590.0000000000	1418.3896624546
1600.0000000000	1122.6207743231
1610.0000000000	1273.6524647955
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1630.0000000000	188.0584418743
1640.0000000000	172.4738981652
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1730.0000000000	43.5057721133
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1800.0000000000	6.7184116257
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1990.0000000000	0.0003766332
2000.0000000000	0.0004544279
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2180.0000000000	0.0007569651
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