Electronic Supplementary Material (ESI) for Dalton Transactions.

Electronic Supporting Information

Slow-targeted release of a ruthenium anticancer agent from vitamin B_{12} functionalized marine diatom microalgae

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Cobalamin derivatives synthesis and characterization

Vitamin B_{12} derivative (B_{12}-**2**)

The cyanide upper part of the vitamin B_{12} was modified in order to label the molecule with a fluorescent dye. For this purpose a FAM azide, 6-isomer supplied from Lumiprobe Life Science Solutions was linked to the vitamin B_{12} through a 1,4-DIETHYNYLBENZENE bridge. The vitamin B_{12} was reacted under conditions previously described by Gryko et al. (reference 16 in manuscript) to give the B_{12}-**2** (see in Scheme 1).

^{1}H NMR (500 MHz, MeOD-[d4]): δ = 7.23 (s, 1H), 7.20 (s, 1H), 7.16 (d, J = 8.3 Hz, 1H), 6.79 (d, J = 8.3 Hz, 1H), 6.62 (s, 1H), 6.19 (d, J = 2.65 Hz, 1H), 5.98 (s, 1H), 4.81-4.78 (m, 2H), 4.63-4.55 (m, 2H), 4.41-4.31 (d, J = 10.45 Hz, 1H), 4.26-4.18 (m, 3H), 3.70-3.62 (m, 9H), 3.62-3.56 (m, 4H), 3.53 (m, 2H), 3.45 (s, 1H), 3.28-3.15 (m, 4H), 3.11 (t, J = 6.40 Hz, 2H), 2.95 (dd, J = 8.50, 5.60 Hz, 1 H), 2.83 (q, J = 5.4 Hz, 1H), 2.61-2.52 (m, 12H), 2.52-2.38 (m, 5H), 2.33 (d, J = 13 Hz, 1H), 2.29 (s, 1H), 2.28 (s, 3H), 2.26-2.18 (m, 1H), 2.13-2.05 (m, 1H), 2.03 (s, 1H), 2.01 (s, 1H), 2.00-1.87 (m, 6H), 1.85 (s, 1H), 1.84-1.82 (m, 1H), 1.80-1.70 (m, 3H), 1.47 (s, 3H), 1.35 (s, 3H), 1.34-1.31 (m, 1H), 1.30 (s, 3H), 1.24 (d, J = 6 Hz, 3H), 1.20-1.14 (m, 1H), 1.12 (s, 3H), 0.51 (s, 1H) ppm; ^{13}C NMR (125 MHz, MeOD-[d4]): δ = 179.9, 178.2, 177.6, 177.5, 176.9, 176.01, 176.97, 175.6, 175.0, 174.4, 174.0, 166.4, 165.9, 158.8, 143.6, 138.8, 135.1, 133.4, 132.7, 131.9, 131.6, 128.3, 121.0, 118.7, 111.9, 108.2, 104.9, 102.6, 95.4, 88.0, 86.2, 84.1, 81.25, 81.20, 79.5, 75.7, 75.2, 73.6, 73.5, 71.5, 71.1, 71.0, 70.7, 70.4, 69.5, 64.5, 59.8, 56.9, 56.6, 55.2, 52.2, 46.43, 46.40, 44.0, 43.3, 40.2, 39.9, 39.0, 36.4, 35.4, 33.3, 33.2, 32.7, 32.6, 32.0, 31.0, 29.6, 28.2, 27.5, 27.4, 20.9, 20.4, 20.3, 20.16, 20.13, 20.0, 17.5, 17.1, 16.4, 16.2 ppm; HRMS (ESI+): $[M+2Na]^{2+}$ = 872.8699, calculated for $C_{83}H_{115}Co_1N_{15}O_{18}P_1Na_2$ = 872.8697.

Vitamin B_{12} derivative (B_{12}-**3**)

For this purpose, B_{12}-**2** was coupled by click reaction to the FAM azide dye. 20mg of B_{12}-**2** (13.8mmol) and 4.1mg of FAM azide dye were solubilize in 0.65ml DMF. Afterwards, 0.5mg of CuSO4 (0.2eq) and 2.5mg of TBTA were dissolved in 0.35ml H2O before being added to the reaction mixture. Finally, 2.5mg of Vitamin C (ascorbic acid) were added to the mixture and reacted overnight at room temperature before recovering the desired product, B_{12}-**3**, with 70% yield.

^{1}H NMR (500 MHz, MeOD-[d4]): δ = 8.08 (s, 1H), 7.94 (s, 2H), 7.50 (s, 1H), 7.38 (s 1H), 7.36 (s, 1H), 7.22 (s, 1H), 7.18 (s, 1H), 6.88 (s, 1H), 6.86 (s, 1H), 6.67 (br s, 2H), 6.62 (s, 1H), 6.52 (br s, 2H), 6.45 (d, J = 9 Hz, 1H), 6.39 (br s, 1H), 6.18 (d, J = 2.80 Hz, 1H), 5.97 (s, 1H), 5.10 (s, 1H), 5.65-5.56 (m, 1H), 4.51 (s, 3H), 4.46 (dd, J = 6.16 Hz, 1H), 4.44-4.31 (m, 1H), 4.22 (br s, 3H), 3.85 (br s, 3H), 3.70-3.48 (m, 16H), 3.46-3.40 (m, 2H), 3.20 (q, J = 7.40 Hz, 8 H), 3.10 (t, J = 6.34 Hz, 2H), 2.93 (dd, J = 8.45, 6.0 Hz, 1H), 2.80 (qt, J = 6.0 Hz, 1H), 2.69 (s, 2H), 2.63-2.54 (m, 6H), 2.54-2.49 (m, 6H), 2.49-2.36 (m, 5H), 2.28 (s, 3H), 2.27 (s, 3H), 2.25-2.15 (m, 3H), 2.12-1.87 (m, 4H), 1.85 (s, 3H), 1.83-1.67 (m, 4H), 1.46 (s, 3H), 1.35 (s, 3H), 1.32-1.26 (m, 18H), 1.22 (d, J = 5.55 Hz, 3H), 1.17 (s, 3H), 1.15 (s, 3H), 0.89 (t, J= 6.5 Hz, 1H), 0.50 (s, 3H); HRMS (ESI+): $[M+H+Na]^{2+}$ = 1090.9403, calculated for $C_{107}H_{134}Co_1N_{19}O_{24}P_1Na_1$ = 1090.9407

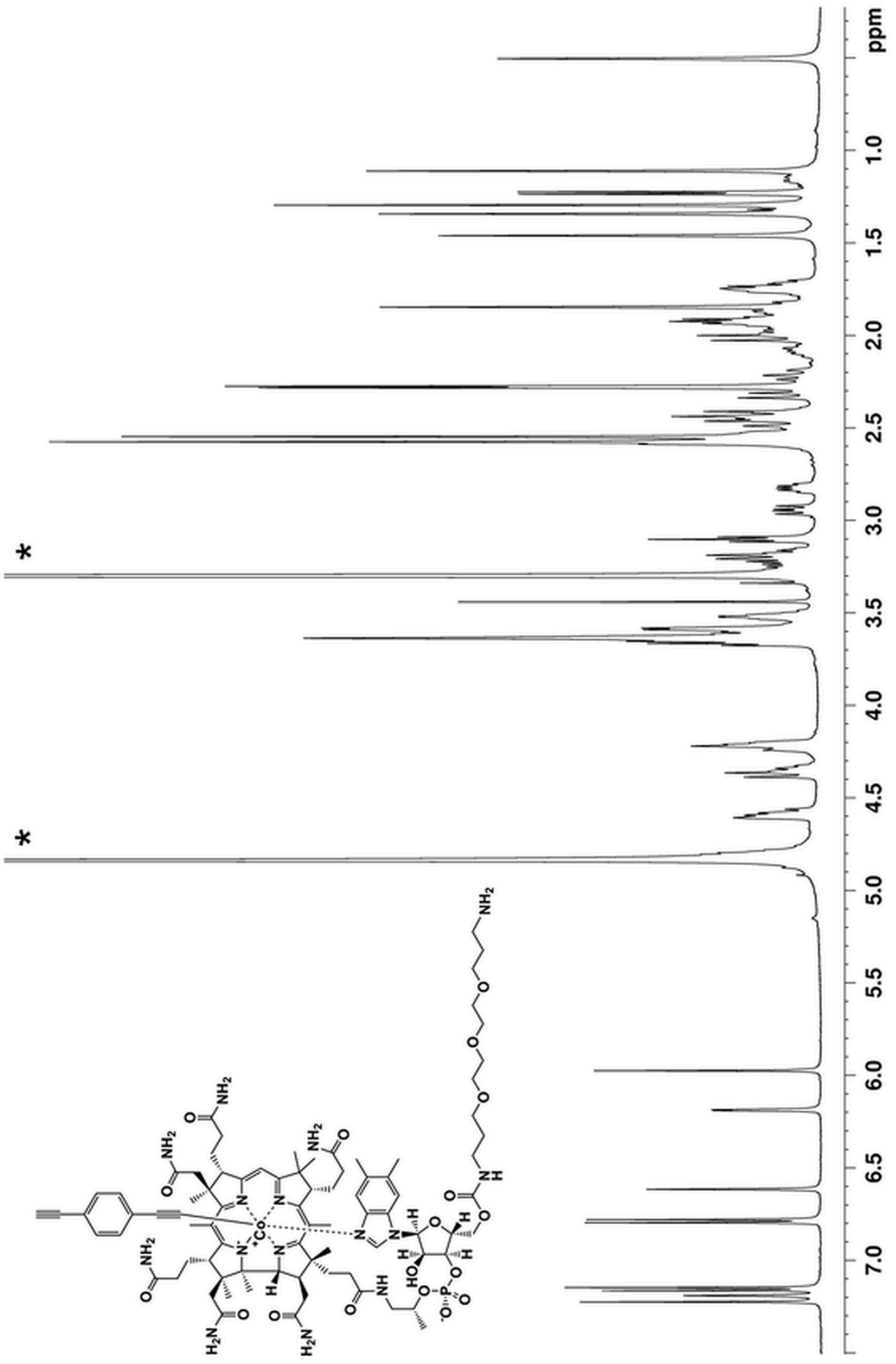


Fig.S1. 500 MHz ^{1}H-NMR of B_{12}-**2** (in MeOD-d4, ✱= solvent signal)

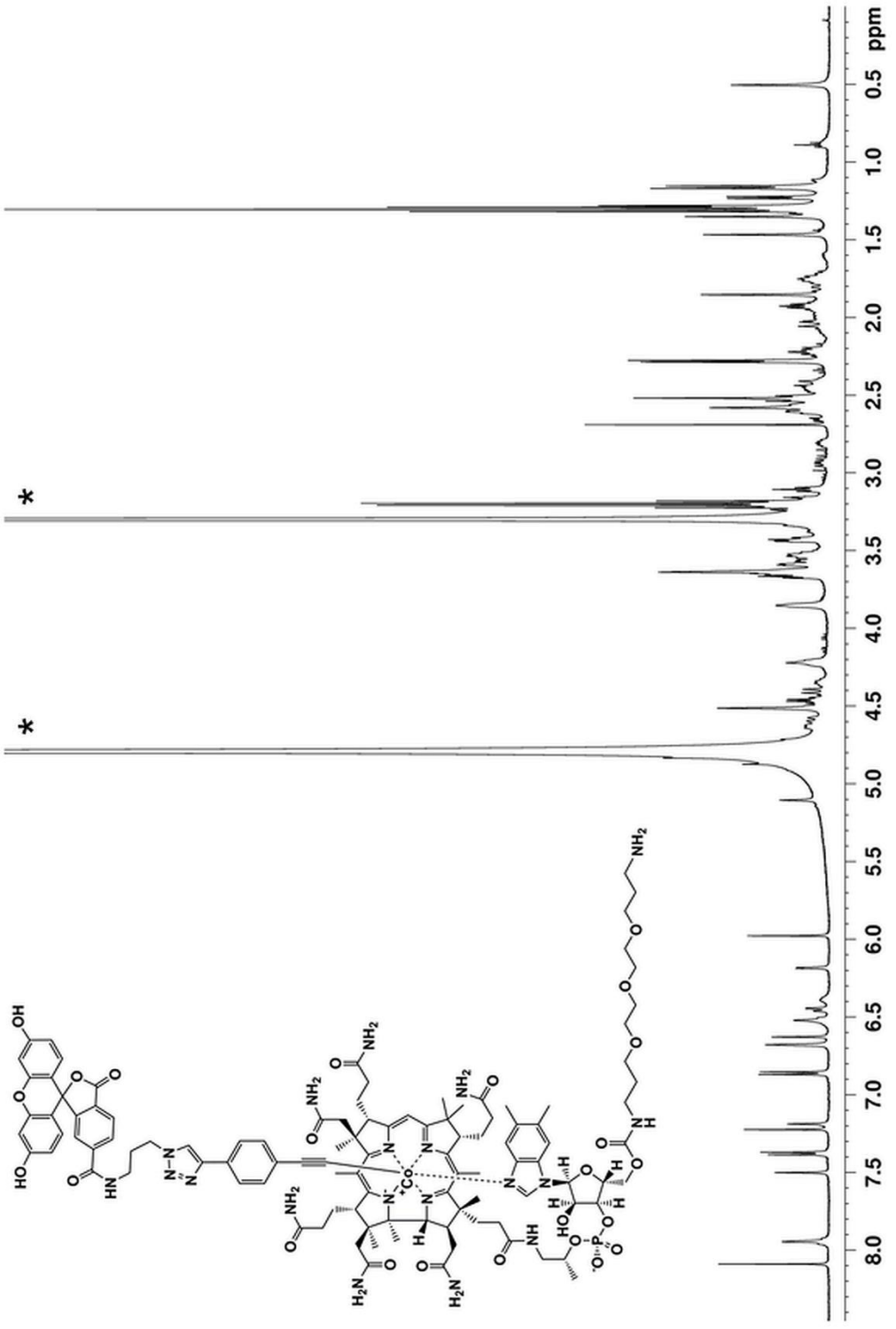


Fig.S2. 500 MHz ^{1}H-NMR of B_{12}-**3** (in MeOD-d4, ✱= solvent signal)

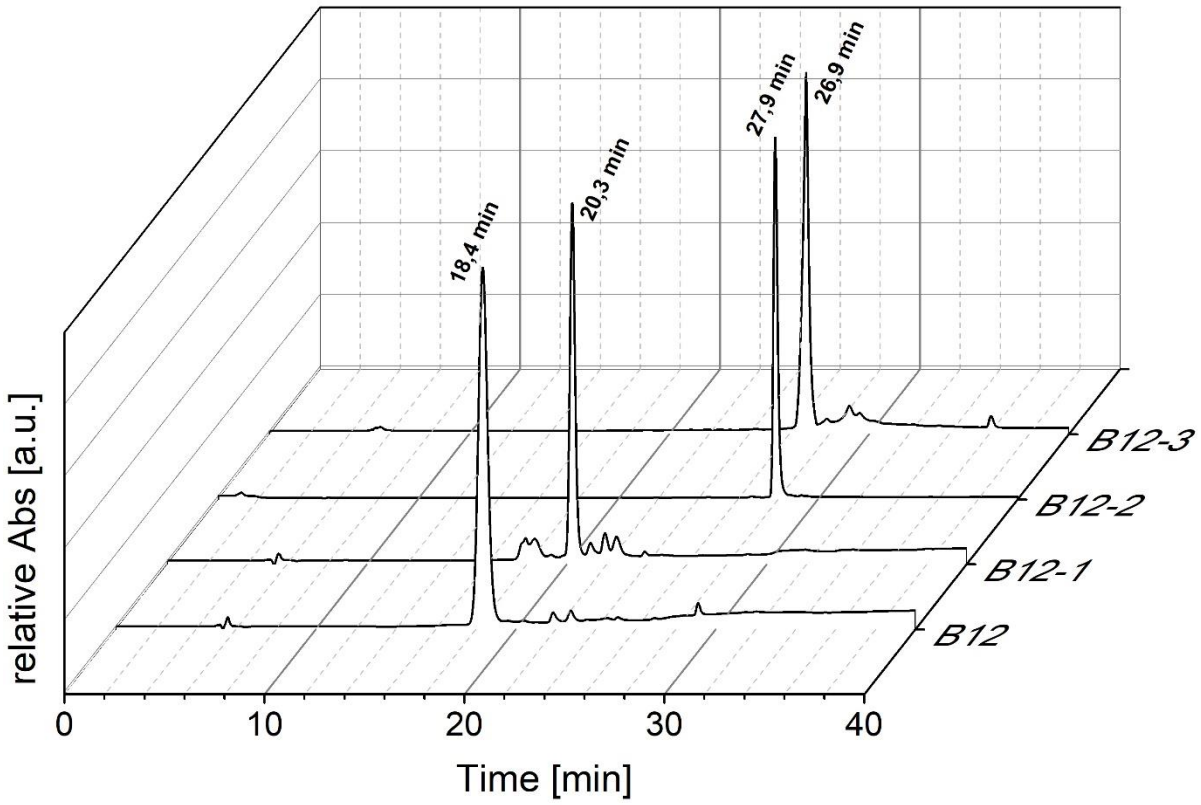


Fig.S3. HPLC chromatograms (B_{12}, B_{12}-**1**, B_{12}-**2**, B_{12}-**3**).

The HPLC analyses were done on a Macherey-Nagel Nucleodur C18 HTec column (5 µm particle size, 250 × 4.6 mm). Aqueous trifluoroacetic acid 0.1% solution and pure methanol were respectively used as solvents (A) and (B). The compounds were separated using the following gradient: 0–5 min (75% A), 5–35 (75% A → 0% A), 35–45 min (100% B), the flow rate set to 0,5 mL min^{-1} and detected at 265 nm. The retention times for the B_{12} and his derivatives B_{12}-**1**, B_{12}-**2** and B_{12}-**3** were respectively 18.4, 20.3, 27.9 and 26.9 min.

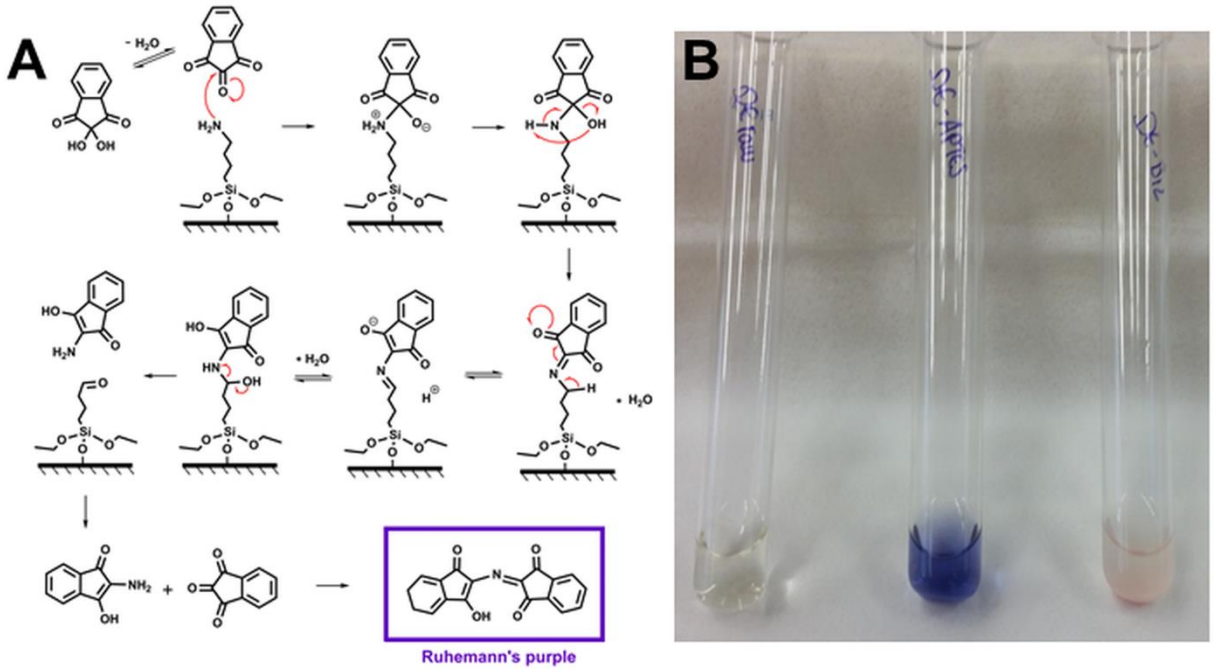


Fig.S4. Ninhydrin test to check surface functionalization.

(A) Scheme of the Ninhydrin dimerization in the presence of primary amines at the surface of silica dioxide. (B) From left to right, Picture of the unmodified DEMs, APTES functionalized DEMs and B_{12} modified DEMs in few milliliters of a staining solution (3.5mg/ml ninhydrin in pure ethanol). If primary amines are present, the solution turn blue-purple, as visible in the middle sample, the suspension of APTES modified DEMs.

The ninhydrin revelation test was performed to assess the successful functionalization of the DEMs surface. Three test tubes were loaded with unmodified DEMs, APTES modified DEMs and DEMs-B_{12}-**1** (from left to right, Figure S3B). After staining with a fresh ninhydrin solution, these three test tubes showed colorations of limpid-incolor, blue-purple and limpid-incolor with reddish glints respectively. This result give the evidence that the surface of DEMs was firstly modified with APTES before being further functionalized with B_{12}-**1** since all the amines were reacted to give amide bonds.

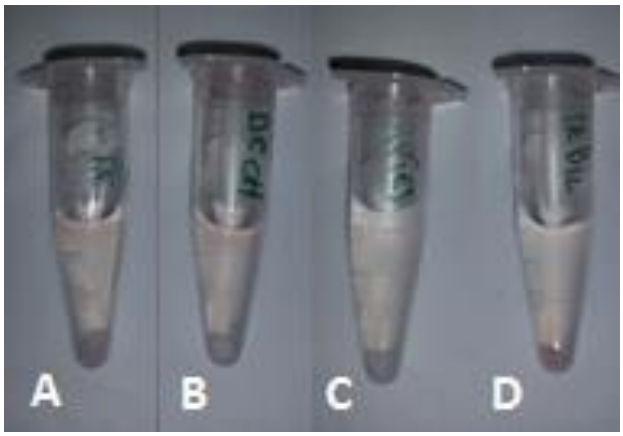


Fig.S5. Release of $[Ru((Et_2N)_2bpy)_3]Cl_2$ in PBS pH 7.4.

From left to right, unmodified (A), hydroxylated (B), APTES functionalized (C) and B_{12} functionalized DEMs (D). Release in PBS buffer pH 7.4 with 1%EtOH. Reddish coloration visible on the wall of the eppendorfs after centrifugation, the DEMs lay on the bottom.

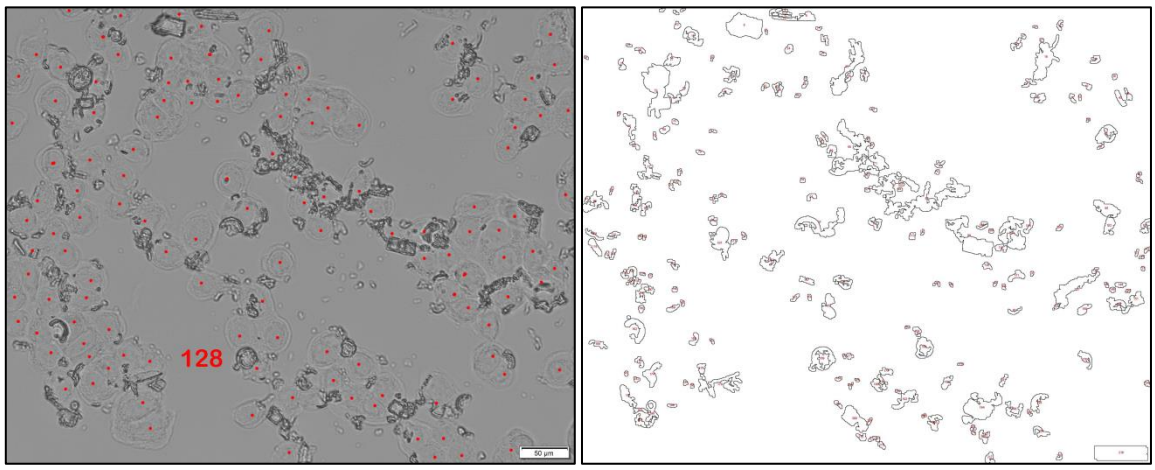


Fig.S6. Representative images. DEMs pieces and cells counting.

Representative Bright field image of the colorectal cancer HT-29 cell line immersed 1h with 200 ug mL^{-1} DEMs-B_{12}-**1** before being deeply washed with fresh media. Left, cells counting with photoshop (red dots). Right, DEMs pieces counting with ImageJ.

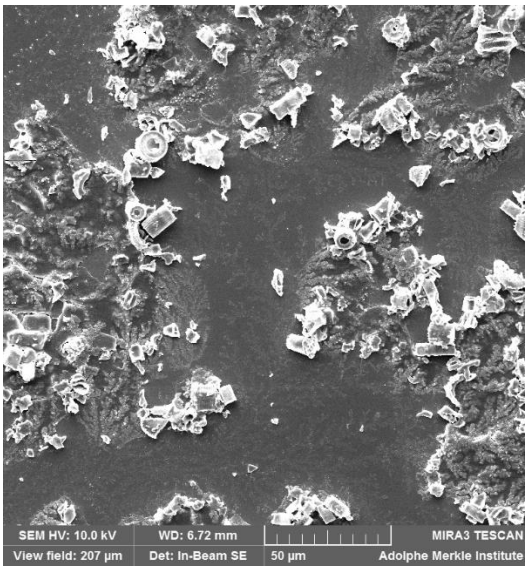


Fig.S7. SEM image of H cells exposed to DEMs-B12-1.

Representative image of MCF-7 cells immersed 1h with 200 ug mL^{-1} DEMs-B_{12}-**1** before being deeply washed with fresh media. The typical shape of the cylindrical diatoms are clearly identified.

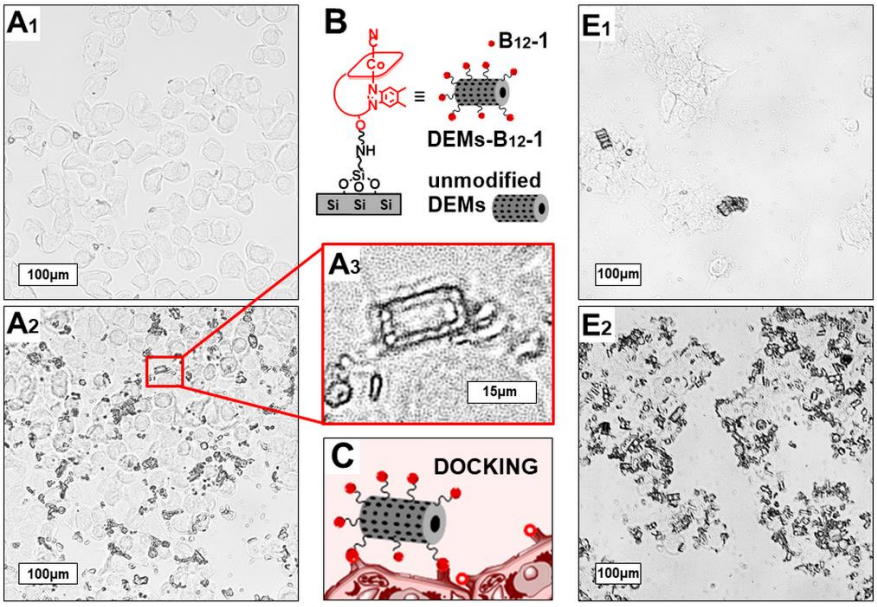
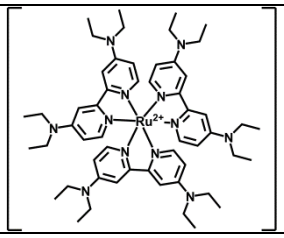
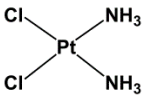
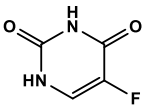


Fig.S8. Bright field images of colorectal cancer cell line HT-29 exposed to: (A1) 200 µg mL-1 of unmodified DEMs; (A2,3) 200 µg mL-1 of DEMs-B_{12}-**1**. (B) Scheme of the DEMs modification by B_{12} bonding. (C) Principle of DEMs-B_{12}-**1** docking to cancer cells. Bright field

images of breast cancer cell line MCF-7 exposed to: (E1) to 200 ug mL-1 of unmodified DEMs; (E2) to 200 µg mL-1 of DEMs-B_{12}-**1**.

Table 1. Physicochemical properties of drug candidates. **4**, Cisplatin and 5-FU were used as drug candidates. Data obtained from Zava et al.[1], Dasari & Tchounwou[2] and Yang et al.[3]

Name	**Ruthenium(II), tris(N,N,N',N'-tetraethyl[2,2'-bipyridine]-4,4'-diamine-N1,N1')-, dichloride**	**Cisplatin**	**5-Fluorouracil**
Chemical formula **Molecular weight g mol^{-1}**	$C_{54}H_{78}N_{12}Ru \cdot 2\,Cl$ 1067.25	$Cl_2H_6N_2Pt$ 301.1	$C_4H_3FN_2O_2$ 130.08
Chemical structure			
Water solubility **pK_a** **log$P_{oct/w}$ (pH 7)**	Insoluble 5.8 0.55	2,53 g/L at 25 °C 6.6 -2.19	12.2 g/L at 20 ºC 8.0 -0.89
IC_{50} [µM] A2780 A2780cisR MCF-7	 >1 >1 -	 4.9 resistant 22.6	 2.0 - 476
Loading degree in DEMs [%] unmodified DEMs DEMs-B_{12}-**1**	 1.2 1.6	 7.4 6.3	 7.3 9.9

[1] Olivier Zava et al., "A Cytotoxic Ruthenium Tris(Bipyridyl) Complex That Accumulates at Plasma Membranes," *ChemBioChem* 10, no. 11 (2009): 1796–1800, https://doi.org/10.1002/cbic.200900013.
[2] Shaloam Dasari and Paul Bernard Tchounwou, "Cisplatin in Cancer Therapy: Molecular Mechanisms of Action," *European Journal of Pharmacology* 0 (October 5, 2014): 364–78, https://doi.org/10.1016/j.ejphar.2014.07.025.
[3] Wanjuan Yang et al., "Genomics of Drug Sensitivity in Cancer (GDSC): A Resource for Therapeutic Biomarker Discovery in Cancer Cells," *Nucleic Acids Research* 41, no. D1 (2013): D955–61, https://doi.org/10.1093/nar/gks1111.