## SUPPLEMENTARY INFORMATION

# Biodialyzed Palladium catalysis enables bioorthogonal drug release to control epithelial progenitor cell differentiation

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# Contents

# 1. Synthesis.

*Materials and equipment.*

All chemicals and reagents were purchased from commercial sources and used without further purification unless otherwise specified. Ascorbic acid was purchased from Scientific Laboratory Supplies, DMF, sodium tetrachloropalladate (II), glutaraldehyde, sodium acetate trihydrate, triphosgene, 2-butyn-1-ol, 3-phenyl-2-propyn-1-ol, diisopropylethylamine and triethylamine were purchased from Sigma Aldrich, gold(III) chloride was purchased from Acros. Lysozyme (HEWL chicken) lyophilized powder (protein ≥90 %, ≥40,000 units/mg protein) was purchased from Sigma Aldrich.

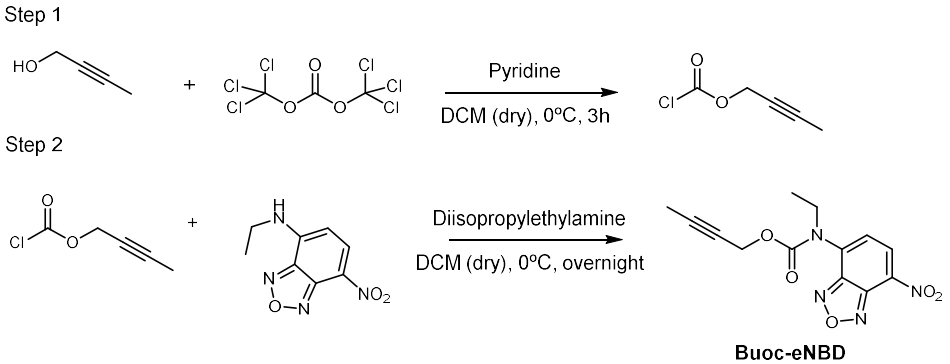
TLC silica gel plates Si 60 $F_{254}$ on aluminum from Merck were used for TLC analysis and preparative-TLC. Flash column chromatography was done using silica gel (220 – 440 mesh).

Analytic LC-MS data was obtained by using Agilent 1260 infinity II system equipped with an InfinityLab Poroshell 120 with binary mixtures made of A (0.1% formic acid, 99.9% $H_2O$), B (0.1% formic acid, 99.9% MeOH) and C (0.1% formic acid, 99.9% MeCN) as a mobile phase (flow = 1.0 mL/min) in a starting linear gradient as described.

NMR spectra were recorded at 300 K temperature on a 500 MHz Bruker Avance III spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the solvent peak.

High resolution mass spectrometry (electrospray ionization) was performed by the University of Edinburgh technician supported mass spectrometry service, using a Bruker miroTOF spectrometer. All samples were ionized via electrospray ionization.

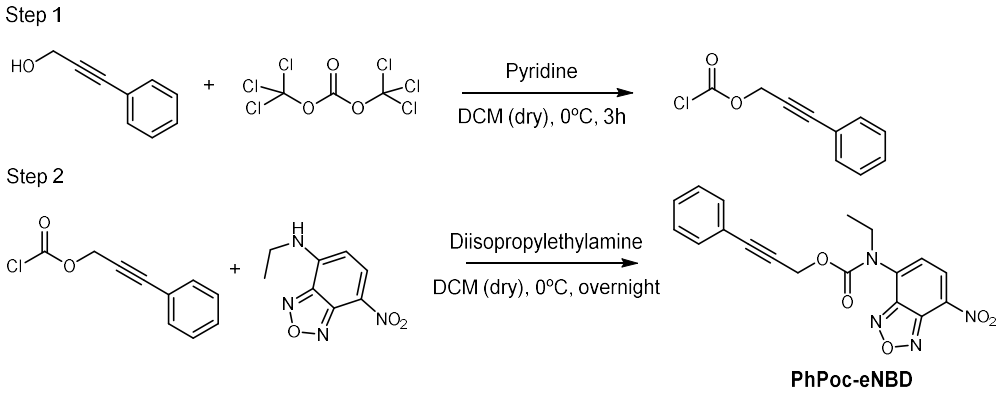
*Synthesis of* ***Buoc-eNBD***.




(*Step 1*) Under nitrogen atmosphere, to a of solution of triphosgene (0.085 g, 0.285 mmol, 0.5 equiv.) in dry dichloromethane (DCM) (0.5 mL), a solution of 2-butyn-1-ol (0.04 g, 0.571 mmol, 1 equiv.) and pyridine (0.046 mL, 0.571 mmol, 1 equiv.) in dry DCM was added dropwise while stirring at 0 ºC.[1] The mixture was stirred at 0 ºC for 3 hours. (*Step 2*) Under nitrogen atmosphere, $NaHCO_3$ (0.238 g, 1.14 mmol, 2 equiv.) was added to a solution of eNBD (0.041 g, 0.382 mmol, 0.67 equiv.) in dry DCM (0.5 mL) to generate a suspension. The suspension was then placed in an ice-bath and diisopropylethylamine (0.200 mL, 1.14 mmol, 2 equiv.)

were added while stirring. The solution generated in *Step 1* was then added to the suspension and stirred at 0 ºC overnight. The crude was then diluted with DCM, washed with $NaHCO_3$ (saturated), dried with $Na_2SO_4$ and purified via flash column using mixtures hexane - ethyl acetate to obtain **Buoc-eNBD** as a yellow solid (16.2 mg, 0.04 mmol, 10.5%). $^1$H NMR (500 MHz, Chloroform-d) δ 8.54 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 4.80 (q, J = 2.4 Hz, 2H), 4.16 (q, J = 7.1 Hz, 2H), 1.87 (t, J = 2.4 Hz, 3H), 1.57 (s, 2H), 1.32 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-d) δ 153.60, 147.50, 143.65, 137.86, 133.81, 131.30, 124.46, 84.07, 72.60, 55.19, 45.62, 14.14, 3.68. m/z (ES$^-$-MS) 303.0735 [M-H]$^-$. Anal. calc. for $C_{13}H_{11}N_4O_5$; found 303.0727, Δppm = +/- 2.7.

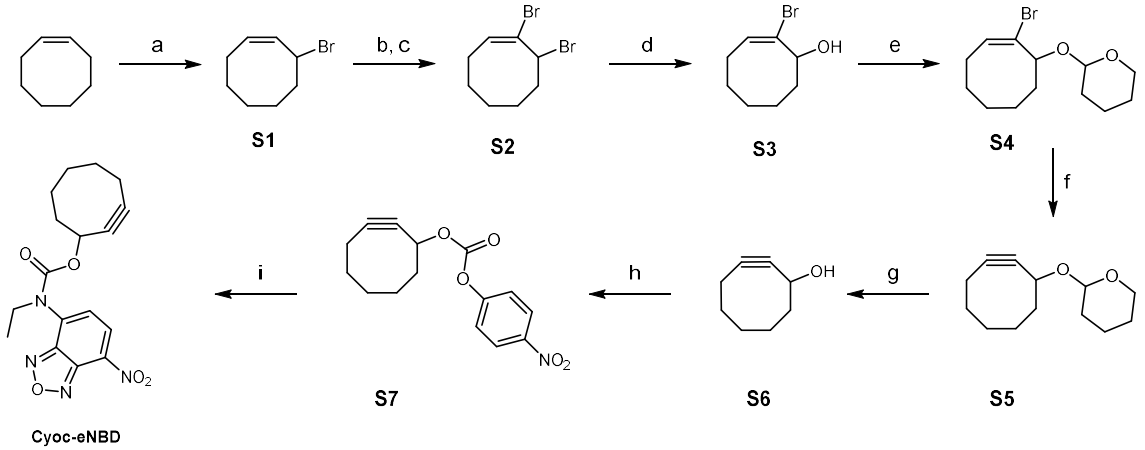
*Synthesis of **PhPhoc-eNBD**.*



**PhPoc-eNBD**

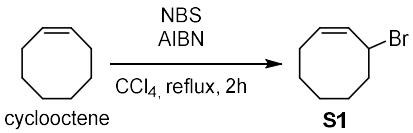
(*Step 1*) Under nitrogen atmosphere, to a of solution of triphosgene (0.042 g, 0.14 mmol, 0.5 equiv.) in dry dichloromethane (DCM) (0.5 mL), a solution of 3-phenyl-2-propyn-1-ol (0.037 g, 0.28 mmol, 1 equiv.) and pyridine (0.023 mL, 0.28 mmol, 1 equiv.) in dry DCM was added dropwise while stirring at 0 ºC.[1] The mixture was stirred at 0 ºC for 3 hours. (*Step 2*) Under nitrogen atmosphere, $NaHCO_3$ (0.117 g, 0.56 mmol, 2 equiv.) was added to a solution of eNBD (0.020 g, 0.19 mmol, 0.67 equiv.) in dry DCM (0.5 mL) to generate a suspension. The suspension was then placed in an ice-bath and diisopropylethylamine (0.100 mL, 0.56 mmol, 2 equiv.) were added while stirring. The solution generated in *Step 1* was then added to the suspension and stirred at 0 ºC overnight. The crude was then diluted with DCM, washed with $NaHCO_3$ (saturated), dried with $Na_2SO_4$ and purified via flash column using mixtures hexane - ethyl acetate to obtain **PhPoc-eNBD** as a yellow solid (6 mg, 0.0164 mmol, 8.7%). $^1$H NMR (500 MHz, Chloroform-d) δ 8.52 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.45 – 7.43 (m, 2H), 7.35 – 7.30 (m, 3H), 5.05 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (126 MHz, Chloroform-d) δ 153.51, 147.55, 143.65, 137.81, 133.94, 131.93, 131.26, 129.00, 128.38, 124.53, 121.87, 87.24, 82.21, 55.20, 45.75, 14.16. m/z (ES$^-$-MS) 365.0891 [M-H]$^-$. Anal. calc. for $C_{18}H_{13}N_4O_5$; found 365.0865, Δppm = +/- 7.2.

*Synthesis of* ***Cyoc-eNBD****.*



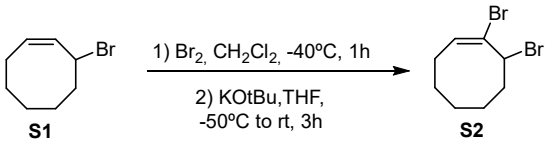

Reagents and conditions: a) NBS, AIBN, $CCl_4$, reflux, 2h,[2] 44%; b) $Br_2$, $CH_2Cl_2$, -40 ºC,[3] 1h; c) KO$^t$Bu, THF, -50 ºC to rt, 3h,[3] 71%; d) $CuSO_4$, DMSO, water, reflux, 2h,[4] 33%; e) THP, Py.TosOH, $CH_2Cl_2$, r.t., 2h,[4] 81%; f) LDA, THF, -78 ºC to rt, 12h,[4] 96%; g) TsOH, MeOH, rt,[4] 25%; h) 4-Nitrobenzyl chloroformate, triethylamine, THF, -10 ºC to rt, 12h,[5] 42%; i) Triethylamine, DMF, rt, 3d. 8%.

*(Step a) Synthesis of 3-bromocyclooctene (****S1****).*




Cyclooctene (24 mL, 185 mmol, 1 equiv.), *N*-bromosuccinimide (NBS, 32 g, 188 mmol, 1 equiv.) and azobisisobutyronitrile (AIBN, 1.5 g, 9.1 mmol, 0.05 equiv.) were dissolved in carbon tetrachloride (90 mL, 0.5 mL/mmol) and stirred under reflux for 2h. The crude mixture was distilled under high vacuum (76-79 °C) to yield 3-bromocyclooctene (**S1**) as a colourless liquid (15.4 g, 8.4 mmol, 44%). $^1$H NMR (500 MHz, $CDCl_3$) δ 5.80 (ddd, *J* = 10.7, 8.3, 1.5 Hz, 1H), 5.72 – 5.52 (m, 1H), 5.08 – 4.85 (m, 1H), 2.30 – 2.17 (m, 2H), 2.17 – 2.06 (m, 1H), 2.07 – 1.95 (m, 1H), 1.77 – 1.64 (m, 2H), 1.64 – 1.45 (m, 2H), 1.46 – 1.27 (m, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 133.4, 129.7, 48.7, 40.9, 28.1, 26.6, 25.7, 23.2.

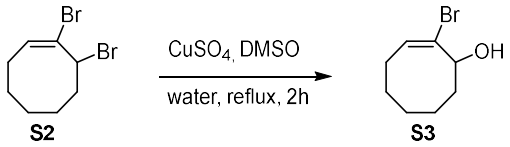
*(Step b,c) Synthesis of 1,8-dibromocyclooct-1-ene (****S2****).*




3-Bromocyclooctene (**S1,** 15.6 g, 82 mmol, 1 equiv.) in $CH_2Cl_2$ (82 mL, 1 mL/mmol) was cooled to –40 °C for the dropwise addition of bromine (4.3 mL, 84 mmol, 1 equiv.) in $CH_2Cl_2$ (55 mL, 0.3 mL/mmol) over 30 min. THF (41 mL, 0.5 mL/mmol) was added, and the solution cooled further to –50 °C, for the addition of

KO$^t$Bu (13.9 g, 124 mmol, 1.5 equiv.) before slowly warming to ambient temperature for 3h. The reaction was cooled to 0 °C and quenched with water (100 mL) and the aqueous phase extracted with $Et_2O$ (2 × 100 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude compound was purified via flash column using permanganate for visualization (100% *n*-Hex) to yield 1,8-dibromocyclooct-1-ene (**S2**) as a colourless oil (15.7 g, 58.6 mmol, 71%). $R_f$ (*n*-Hex) = 0.45. $^1$H NMR (500 MHz, $CDCl_3$) δ 6.24 (t, *J* = 8.8 Hz, 1H), 5.20 (dd, *J* = 12.0, 4.9 Hz, 1H), 2.31 – 2.15 (m, 3H), 2.03 (ddt, *J* = 13.5, 11.7, 5.0 Hz, 1H), 1.92 – 1.64 (m, 3H), 1.48 – 1.21 (m, 3H).

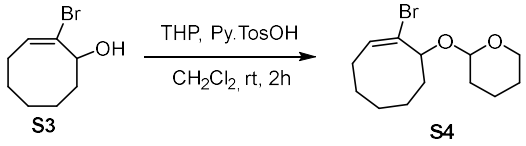
*(Step d) Synthesis of 2-bromocyclooct-2-enol (**S3**).*



S2 S3

1,8-Dibromocyclooct-1-ene (**S2**, 15.7 g, 59 mmol, 1 equiv.) was dissolved in water (34 mL, 0.6 mL/mmol) and DMSO (50 mL, 0.9 mL/mmol) and copper sulfate pentahydrate added (18.5 g, 74 mmol, 1.3 equiv.). The reaction was heated at reflux for 2h, cooled to room temperature diluted with water (60 mL). The aqueous phase was extracted with EtOAc (60 mL) and washed with water (5 × 60 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. The crude was purified via flash column using permanganate for visualization (10% EtOAc / cyhex) to yield 2-bromocyclooct-2-enol (**S3**) as a colourless oil (4.0 g, 19.5 mmol, 33%). $R_f$ (10% EtOAc / cyhex) = 0.36. $^1$H NMR (500 MHz, $CDCl_3$) δ 6.20 (td, *J* = 8.7, 1.0 Hz, 1H), 4.67 (ddd, *J* = 10.8, 4.8, 1.0 Hz, 1H), 2.29 – 2.11 (m, 2H), 2.05 (s, 1H), 1.89 – 1.72 (m, 3H), 1.72 – 1.60 (m, 2H), 1.43 – 1.30 (m, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 132.2, 129.8, 68.8, 36.62, 30.3, 29.0, 26.9, 24.0.

*(Step e) Synthesis of 2-[(2-bromocyclooct-2-en-1-yl)oxy]tetrahydro-2H-pyran (**S4**, mixed diastereomers).*

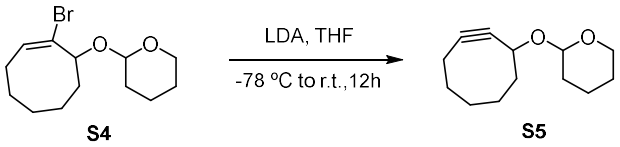


S3 S4

2-Bromocyclooct-2-enol (**S3**, 4.0 g, 9.5 mmol, 1 equiv.), 3,4-dihydropyran (THP, 2.7 mL, 2.5 g, 29.6 mmol, 1.5 equiv.) and pyridinium tosylate (Py.TosOH, 493 mg, 5.9 mmol, 0.3 equiv.) were dissolved in $CH_2Cl_2$ (130 mL, 6.6 mL/mmol) stirred at ambient temperature for 3h. The crude was directly purified via flash column using permanganate for visualization (10% EtOAc / cyhex) to yield 2-[(2-bromocyclooct-2-en-1-yl)oxy]tetrahydro-2*H*-pyran (**S4**) as a colourless oil (4.59 g, 15.9 mmol, 81%). $R_f$ (10% EtOAc / cyhex) = 0.47. $^1$H NMR (500 MHz, $CDCl_3$) δ $^1$H NMR (500 MHz, $CDCl_3$) δ 6.37 – 6.27 (m, 1H), 6.23 – 6.18 (m, 0.15H), 6.18 – 6.13 (m, 0.3H), 4.97 (dd, *J* = 5.0, 2.8 Hz, 0.3H), 4.88 – 4.78 (m, 1H), 4.73 – 4.63 (m, 1H), 4.59 (dd, *J* = 4.8, 2.9 Hz, 1H), 3.97 – 3.86 (m, 1H), 3.55 (dddd, *J* = 10.9, 7.2, 4.1, 2.6 Hz, 2H), 2.31 – 2.11 (m, 3H), 1.95

– 1.84 (m, 2H), 1.84 – 1.61 (m, 11H), 1.61 – 1.48 (m, 5H), 1.48 – 1.25 (m, 5H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 135.0, 131.9, 127.7, 127.6, 97.2, 96.3, 72.6, 71.0, 68.8, 62.9, 62.1, 33.8, 33.4, 30.7, 30.6, 30.4, 30.3, 29.0, 28.9, 27.1, 26.9, 25.6, 25.5, 23.7, 23.6, 22.7, 19.7, 19.0.

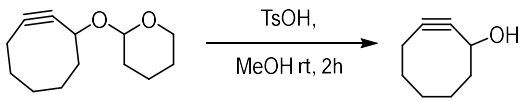
*(Step f) Synthesis of 2-(2-cyclooctyn-1-yloxy)tetrahydro-2H-pyran (**S5**, mixed diastereomers).*




**S4** **S5**

LDA solution (2.8 mL, 2 M in THF, 5.6 mmol, 1 equiv.) was diluted in dry THF (7.3 mL, 1.4 mL/mmol) and cooled to -78 ºC, and a 2-[(2-bromocyclooct-2-en-1-yl)oxy]tetrahydro-2*H*-pyran (**S4**, 1.6 g, 5.5 mmol, 1 equiv.) in THF (5.7 mL, 1.1 mL/mmol) was added dropwise, with vigorous stirring. The reaction was left to warm to ambient temperature, stirring overnight. The reaction was quenched with slow addition of water (40 mL) at 0 ºC then extracted into EtOAc (3 × 40 mL) and dried over $MgSO_4$. The crude alkyne was filtered through a plug of silica (10% EtOAc / *n*-Hex) and concentrated in vacuo to yield 2-(2-cyclooctyn-1-yloxy)tetrahydro-2*H*-pyran (**S5**, 1.1 g, 5.3 mmol, 96%) as a colourless oil. $R_f$ (10% EtOAc / cyhex) = 0.45 / 0.40 (two spots). $^1$H NMR (500 MHz, MeOD) δ 6.34 (t, *J* = 8.7 Hz, 1H), 6.15 (t, *J* = 8.7 Hz, 1H), 4.85 (dd, *J* = 6.3, 2.9 Hz, 2H), 4.70 (dd, *J* = 10.6, 4.9 Hz, 1H), 4.58 (dd, *J* = 4.3, 2.9 Hz, 1H), 3.91 (m 2H), 3.61 – 3.44 (m, 2H), 2.34 – 2.07 (m, 4H), 1.86 (m, 4H), 1.72 (m, 10H), 1.66 – 1.44 (m, 8H), 1.46 – 1.27 (m, 6H). $^{13}$C NMR (126 MHz, MeOD) δ 135.2, 132.0, 127.3, 126.9, 97.3, 96.0, 72.9, 70.8, 62.2, 61.8, 33.4, 33.1, 30.3, 30.2, 30.1, 30.0, 28.7, 28.5, 26.9, 26.6, 25.3, 25.2, 23.4, 23.2, 19.0, 18.6.

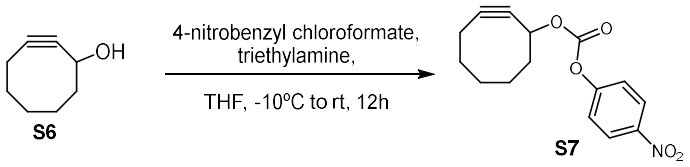
*(Step g) Synthesis of 2-cyclooctyn-1-ol (**S6**).*




**S5** **S6**

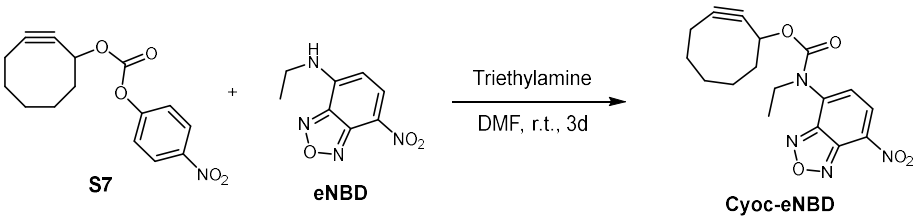
2-(2-cyclooctyn-1-yloxy)tetrahydro-2*H*-pyran (**S5**, 1.1 g, 5.3 mmol, 1 equiv.) was dissolved in methanol (8 mL, 1.5 mL/mol) and *p*-toluenesulfonic acid (TsOH, 0.9 mmol, 0.17 equiv.) was added. The reaction was stirred 2h at ambient temperature. Water (50 mL) was added and the aqueous phase extracted with EtOAc (3 × 60 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude alcohol was purified by via flash column using permanganate for visualization (20% EtOAc / *n*-Hex) to yield 2-cyclooctyn-1-ol (**S6**) as a colourless oil (163 mg, 1.3 mmol, 25%). $R_f$ (20% EtOAc / *n*-Hex) = 0.35. $^1$H NMR (400 MHz, $CDCl_3$) δ 4.48 (dq, *J* = 7.1, 3.8 Hz, 1H), 2.35 – 2.10 (m, 3H), 2.04 – 1.85 (m, 3H), 1.84 – 1.75 (m, 1H), 1.70 – 1.58 (m, 2H), 1.63 – 1.47 (m, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 99.8, 94.1, 64.7, 45.3, 34.4, 29.8, 25.9, 20.7.

(*Step h) Synthesis of cyclooct-2-yn-1-yl 4-nitrophenyl carbonate (**S7**).*



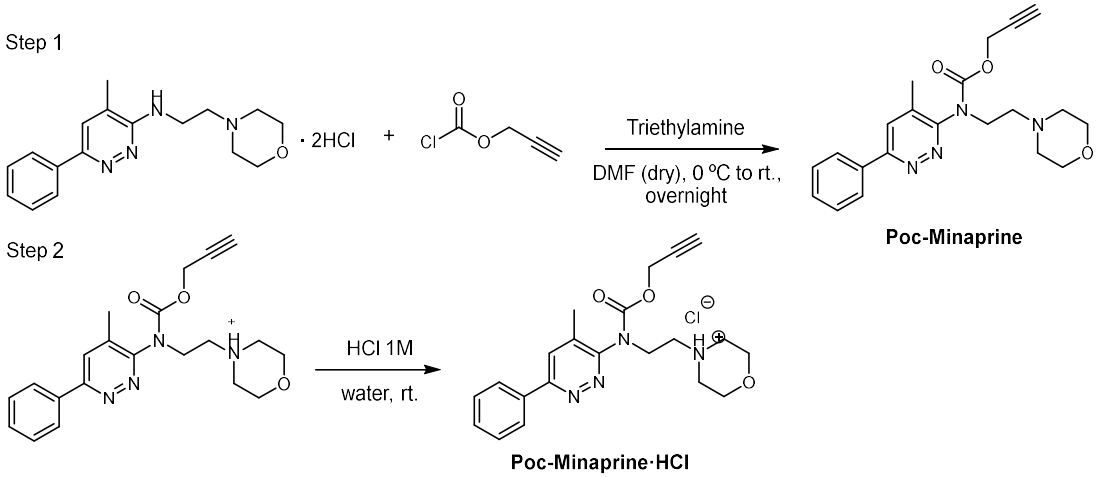

A solution of 2-cyclooctyn-1-ol (**S6**, 70 mg, 0.56 mmol, 1 equiv.) and triethylamine (95 µL, 0.7 mmol, 1.3 equiv.) in THF (2.8 mL, 5 mL/mmol) was added dropwise to a stirred solution of 4-nitrophenyl chloroformate (340 mg, 1.7 mmol, 3 equiv.) in THF (0.6 mL, 1 mL/mmol) at 0 ºC. After addition was complete, the reaction was slowly warmed to ambient temperature and stirred overnight. Solvent was removed in vacuo and the crude product purified by preparative-TLC (10% EtOAc / *n*-Hex) to yield cyclooct-2-yn-1-yl 4-nitrophenyl carbonate (**S7**) as a yellow oil (69 mg, 0.24 mmol, 42%). $R_f$ (10% EtOAc / *n*-Hex) = 0.42. $^1$H NMR (500 MHz, $CDCl_3$) δ 8.33 – 8.22 (m, 2H), 7.47 – 7.37 (m, 2H), 5.40 – 5.29 (m, 1H), 2.35 (dtd, *J* = 17.0, 6.4, 1.7 Hz, 1H), 2.31 – 2.18 (m, 3H), 2.03 – 1.93 (m, 2H), 1.93 – 1.75 (m, 2H), 1.73 – 1.61 (m, 2H).

*(Step i) Synthesis of **Cyoc-eNBD**.*




Cyclooct-2-yn-1-yl 4-nitrophenyl carbonate (28 mg, 0.096 mmol, 1 equiv.), (N-ethyl-7-nitrobenzo[c][1,2,5]oxadiazol-4-amine (**eNBD**)[6] (30 mg, 0.14 mmol, 1.5 equiv.) and triethylamine (15 µL, 0.15 mmol, 1.5 equiv.) were stirred in DMF (0.5 mL) for 3 days at ambient temperature. Solvent was removed in vacuo and compound purified by preparative-TLC (30% EtOAc/*n*-Hex) to yield **Cyoc-eNBD** as a yellow solid (2.8 mg, 0.007 mmol, 8%). $^1$H NMR (500 MHz, DMSO) δ 8.73 (d, *J* = 8.0 Hz, 1H, ArH), 7.75 (d, *J* = 8.0 Hz, 1H, ArH), 5.34 – 5.25 (m, 1H, O-CH), 3.92 (q, *J* = 7.1 Hz, 2H, $CH_2$ Et), 2.23 (dtd, *J* = 17.0, 6.4, 1.9 Hz, 1H, $CH_2$), 2.15 (dtd, *J* = 16.9, 6.2, 2.4 Hz, 1H, $CH_2$), 2.10 – 2.01 (m, 1H, $CH_2$), 1.90 – 1.71 (m, 3H, $CH_2$), 1.62 – 1.39 (m, 4H), 1.18 (t, *J* = 7.1 Hz, 3H, $CH_3$, Et). $^{13}$C NMR (126 MHz, DMSO) δ 152.7, 147.9, 143.7, 136.9, 133.8, 133.1, 125.9, 102.4, 90.6, 68.5, 45.3, 41.1, 33.7, 29.0, 25.5, 19.9, 13.7. HRMS (ESI) m/z: $[M+Na]^+$ calculated $C_{17}H_{18}N_4O_5Na$: 381.11694, found = 381.1159, Δppm = +/- 2.73, $[M+-H]^-$ calculated $C_{17}H_{18}N_4O_5$ : 357.12044, found = 357.1216 Δppm = +/- 3.25.

*Synthesis of* ***Poc-Minaprine·HCl****.*




(*Step 1*) Under nitrogen atmosphere, Minaprine dihydrochloride (0.1 g, 0.270 mmol, 1 equiv.) was dissolved in 1 mL of dry DMF by adding triethylamine (0.131 mL, 0.943 mmol, 3.5 equiv.) while stirring. The solution was placed in an ice-bath and propargyl chloroformate (0.032 mL, 0.324 mmol, 1.2 equiv.) was added dropwise. The mixture was allowed to reach room temperature and stirred overnight. The reaction was then quenched by adding a few drops of water and the solvent was removed under vacuum. The crude was dissolved in ethyl-acetate (10 mL) and washed with water and brine, and furtherly dried over $MgSO_4$. Solvent was removed in vacuo and product purified via flash chromatography eluting with DCM:MeOH (9:1). Poc-Minaprine was obtained as a white solid. (*Step 2*) At room temperature, HCl 1 M was added dropwise over a stirred suspension of **Poc-Minaprine** in 5 mL of water until a clear solution was obtained. The excess of acid was then removed under vacuum, and the rest of water by freeze drying. **Poc-Minaprine**·HCl was obtained as a white powder (39 mg, 0.094 mmol, 34.6%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 10.98 (s, 1H), 8.37 (s, 1H), 8.27 – 8.00 (m, 2H), 7.76 – 7.48 (m, 3H), 4.84 (s, 2H), 3.99 (d, J = 12.1 Hz, 2H), 3.81 (t, J = 12.2 Hz, 2H), 3.52 (d, J = 12.1 Hz, 4H), 3.17 (s, 2H), 2.35 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 158.52, 157.09, 153.52, 138.17, 135.64, 130.81, 129.61, 128.13, 127.44, 78.82, 78.68, 63.78, 61.36, 54.30, 51.88, 43.45, 17.16. (ESI) m/z: $[M+H]^+$ calculated $C_{21}H_{25}N_4O_3$: 381.4555, found = 381.4511, Δppm = +/- 0.28.

## 2. Catalyst production.

*Cross-Linked Lysozyme Crystals.*

Lysozyme crystals (LCs) of 250 μm size were prepared following a gelled batch of agarose procedure, inducing the nucleation via seeding. LCs seeds were prepared following a standard batch crystallization procedure by mixing 150 mg·mL$^{-1}$ stock solution of lysozyme in 50 mM sodium acetate buffer at pH 4.5, with NaCl to reach 40 mg/mL of protein and 2.7% (w/v) NaCl final concentrations. The mixture was incubated at 20 ºC for one week. The LCs obtained were detached from the walls using a cat whisker and centrifuged at 10,000 RPM for 1 minute. LCs were grinded using a glass rod until no crystalline particles could be distinguished under an

optical microscope. LCs of 250 µm size were prepared by mixing an 1% (w/v) aqueous solution of agarose D5 dissolved at boiling point and then kept at 40 ºC, with solutions of 150 mg·mL$^{-1}$ solution of lysozyme (40 mg·mL$^{-1}$ final) and NaCl (2.7% (m/v) final), both in 50 mM sodium acetate buffer at pH 4.5 at 40 ºC. The crystallization was then triggered by adding 5 µL of seed suspension per 0.5 mL of final mixture. LCs were obtained after 24 h at 20 ºC. The cross-linked lysozyme crystals (CLLCs) were obtained by adding equal volume of 10% (v/v) glutaraldehyde with NaCl 2.7% (w/v) on the top of the gel containing the LC and allowed to diffuse for 48 hours. The supernatant was then removed, and the resulting gel was freeze-dried. The rests from the gel and the salts, were removed from the CLLCs by resuspending the obtained solid in clean water and removing the supernatant and floating solids.

*Pd@CLLCs.*

2 mg of freeze-dried CLLCs were soaked in 250 µL of aqueous solution of $Na_2PdCl_4$ 0.1 M for four days at room temperature. The supernatant was then removed, and crystals rinsed with clean water. The entrapped excess of Pd salt within the crystals was then removed by soaking them in 10 mL of clean water and kept at room temperature for two days (twice). The resulting Pd(II)@CLLCs were then freeze-dried and typically sorted in 26 crystals batches. 200 µL of ascorbic acid 100 mg·mL$^{-1}$ were added to each crystals' batch and kept at 37 ºC for 3 days. The supernatant was then removed and the Pd@CLLCs obtained rinsed with water. Each crystal batch was then soaked in 1 mL of water for two days (twice).

Pd@CLLCs were counted and weighted in triplicate and the average mass per crystal was calculated as 17.2 ± 0.9 µg/crystal.

*Au@CLLCs.*

Empty CLLCs (26) were soaked in a solution of and Au(III) chloride in water (250 µL, 0.1 M), and left undisturbed in darkness for 4 d. The gold solution was then removed Au(III)@CLLCs washed with water (3 × 200 µL, then 2 x 1 mL for 2 d). Water was removed and ascorbic acid (200 µL, 567 mM) was added and the crystals left undisturbed, at 37 ºC, in darkness for 3 d. Ascorbic acid was removed and Au@CLLCs rinsed with water (3 × 200 µL, then 1 mL for 2 d at 37 ºC).

## 3. General procedures.

*XRD – Synchrotron source.*

Lysozyme crystals for X-ray diffraction were obtained from the set of the smallest lysozyme crystals obtained as described above. Crystals were transferred to a cryoprotectant solution containing 15% (v/v) of glycerol in the crystallization solution supplemented with 100 mM of Pd and soaked for less than 30 seconds. Crystals were fished-out with the help of the litho-loop and flash cooled in liquid nitrogen. X-ray diffraction data were

collected at beamline ID23-1 (GEMINI) of the European Synchrotron Radiation Facility (ESRF), Grenoble, France. Reflections were recorded on a Eiger 2 16M CdTe (Dectris) detector. Data were indexed and integrated with XDS,[7] scaled and reduced with AIMLESS[8] of the CCP4 program suite.[9] The 3D structural model was determined by molecular replacement with Molrep[10] using the clean PDB model of Lysozyme (ID. 1IEE[11]). Refinement was done using phenix.refine[12] and Refmac[13] of the CCP4 and PHENIX[14] program suites with water inspection and metal identification done in Coot.[15] The crystallographic data statistics and final model characteristics are provided in **Supp. Table S1**.

**Supplementary Table S1.** XRD synchrotron data collection and refinement statistics.

| | **Lysozyme** |
|---|---|
| Synchrotron/line | ESRF/ID23-1 |
| Resolution range (Å) | 37.4 - 1.79 (1.83 - 1.79) |
| Space group | P $4_3$ $2_1$ 2 |
| Cell dimensions | |
| a, b, c (Å) | 78.49 78.49 37.37 |
| α, β, γ (°) | 90.0 90.0 90.0 |
| Total reflections | 90572 (4728) |
| Unique reflections | 11419 (603) |
| Multiplicity | 7.9 (7.8) |
| Completeness (%) | 99.4 (90.7) |
| Mean I/sigma(I) | 17.1 (1.1) |
| Wilson B-factor | 38.96 |
| R-merge | 0.049 (1.684) |
| CC1/2 | 0.999 (0.481) |
| **Refinement** | |
| R-work/ R-free (%) | 21.29 / 25.05 |
| No. atoms | 1139 |
| macromolecules | 1052 |
| Ligands | 19 |
| Solvent | 68 |
| R.M.S.D. | |
| Bond lengths (Å) | 0.002 |
| Bond angles (°) | 0.43 |
| Ramachandran | |
| favored (%) | 97.64 |
| allowed (%) | 2.36 |
| outliers (%) | 0.00 |
| Average B-factor | 47.09 |
| macromolecules | 46.76 |
| Ligands | 66.28 |
| Solvent | 46.78 |

*Statistics for the highest-resolution shell are shown in parentheses.

*ICP-MS determination of Pd concentration in Pd@CLLCs.*

The palladium content was determined by ICP-MS using a mass spectrometer with plasma torch ionization source and quadrupole ion filter NexION 300D. Prior to measurements, the crystals were mineralized to remove organic matter by adding concentrated $HNO_3$ (2 mL per 100 mg Pd@CLLCs) and heating at 160 °C until complete dryness. Subsequently, $HClO_4$ (1 mL per 100 mg Pd@CLLCs) was added to the dry residue and heated to dryness once more. After that, 4 mL of concentrated $HNO_3$ to the dry residue and cooled until the mixture reached room temperature. Finally, the volume was made up to 100 mL with MiliQ water. The determination of Pd concentration interpolating in a standard calibration resulted in 225 ± 12 mg Pd per g of Pd@CLLC. From these measurements, the approximate Pd amount in each Pd@CLLC was determined to be 3.87 µg Pd, which means that 26 crystals contain approximately 0.1 mg of Pd.

*ICP-MS determination of Pd released from Pd@CLLCs under physiological conditions.*

Pd@CLLCs (between 0-10 per well) were added to a 12-well microplate in 10% FBS in PBS (1 mL). The microplate was kept at 37ºC, 5% $CO_2$ for the duration on the experiment, in a sterile environment. At regular intervals, a sample of media (100 µL) was removed from each well and replenished with fresh PBS. The aliquots were diluted 100 times by adding $HNO_3$ 1.5 M and submitted for ICP-MS analysis as in the previous section. The values of amount of Pd released were calculated by interpolating in a calibration curve and the solution removal and subsequent replenishment was considered for the calculation.

*XPS.*

25-30 Pd@CLLCs crystals (1 $mm^2$ approximated area) were placed on an adhesive copper surface and subjected to XPS analysis. The measurements were performed using a 10 keV $Ar^+_{2000}$, rastered over the surface for 60 s. The analysis was conducted with a 110 µm aperture, FOV1, and a Pass Energy of 40eV.

**Supplementary Table S2.** XPS depth-profiling of Pd/C content as a function of etching time.

| Etching time (min) | Pd/C atomic ratio |
|---|---|
| 1 | 0.012 |
| 5 | 0.013 |
| 10 | 0.014 |
| 15 | 0.015 |
| 20 | 0.017 |
| 25 | 0.017 |
| 30 | 0.017 |

*SEM.*

Images were collected using a FEI Cryogenic Dual Beam-Nova 200 scanning electron microscope (SEM). Wet crystals were placed on a holder and introduced inside the equipment through a cryogenic chamber (cryo-transfer) coupled to the microscope. A focalized ion beam (FIB) was employed to carve a hole on the crystal's surface for furtherly analyze the atomic composition inside the crystals via energy-dispersive X-ray micro-analysis (EDX).

*TEM.*

For sample preparation, the cross-linked crystals were dehydrated with ethanol and embedded in Embed 812 resin. Ultrathin sections (50–70 nm) were prepared using a Reichert Ultracut S microtome (Leica Microsystems GmbH, Wetzlar, Germany) after which the sections were deposited onto copper grids. Transmission Electron Microscopy (TEM) analysis was performed using a Tecnai T20 microscope (Thermo Fisher Scientific) at ELECMI-LMA ICTS Spain) This instrument operates at accelerating voltages of 200 kV and is equipped with a thermionic $LaB_6$ electron gun. The microscope features a SuperTwin® objective lens, providing a spatial resolution of 0.24 nm, which enables high-resolution imaging and detailed structural characterization of the samples. To minimize structural damage to the Pd@CLLCs crystals, the analysis was conducted using a low-electron-dose protocol.

*Biocompatibility of Pd@CLLCs.*

Mouse Embryonic Fibroblasts (MEF) cells were seeded in a 96-well plate format at 3000 cells / well and incubated for 24 h before treatment. Pd@CLLCs crystals were added to 96-well tissue culture inserts (Corning Transwell®, 3.0 μm pore polycarbonate membrane) and soaked in PBS at 37 ºC for 24 h, before treatment. Media from each well was refreshed (230 μL) and the inserts containing Pd@CLLCs (23 and 54 average amount of crystals) were added to the MEF cells, and incubated together 48 h. Inserts containing crystals were removed, and media in each well was replaced with fresh media (130 μL), PrestoBlue cell viability reagent (10% v/v) was added and plate was incubated for 180 min. Fluorescence emission was detected using a Tecan Infinite M plex plate reader (Ex/Em: 560/590 nm). Data was normalized to untreated cells (100% viability) and media (no cells, 0% viability). Experiments were performed in duplicate.

Mouse embryonic fibroblasts (MEF) cells (a kind gift from Professor Javier Cáceres' lab) were cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with serum (10 % FBS) and L-glutamine (2 mM) and incubated at 37 ºC and 5% $CO_2$.

*Uncaging of pro-dyes assays – General procedures.*

250 μM of 20 μM solution **Poc-eNBD** (or **Cyoc-eNBD**, **Buoc-eNBD** or **PhPoc-eNBD**) in 2% v/v DMSO in PBS were added over 26 Pd@CLLCs (or Au@CLLCs) and heated at 37 ºC in a thermoblock for 24 h. Aliquots

of 100 µL were transferred to a 96 well-plate, and the conversion quantified by fluorescence measurements, compared to a calibration curve. Fluorescence emission was detected using a Tecan Infinite M plex and CLARIOstar Plus plate readers (Ex/Em: 440/535 nm). Experiments were performed in triplicate.

- Experiments in presence of FBS: Experiments performed in a sterile environment. The 20 µM solution of **Poc-eNBD** was prepared by diluting 1 mM stock solution of **Poc-eNBD** in DMSO with PBS+10%FBS.
- Recyclability experiments: After each 24 h uncaging cycle, the solution was removed and the crystals rinsed with 100 µL of PBS or PBS+10%FBS (six replicates).
- Uncaging in function of time: For each time cap, fresh crystals and prodye solutions were employed.

*NIR-stimulated uncaging experiments.*

The NIR irradiation set-up consisted of an 808 nm laser module with 400 µm@1m fiber coupling (MDL-III-808-2.5W model, Changchun New Industries Optoelectronics Technology Co., Ltd = CNI laser) connected to a fixed focus collimator (SMA905 model; CNI laser). The laser diode was driven using a variable power supply unit (PSU-III-LED model, CNI laser). The optical fibre was coupled to a micro positioning system. For the irradiation experiments, the tubes containing the samples were placed in a thermostatic water bath at 37 ºC and irradiated with the laser focused on the crystals placed at 9 cm from the source. All irradiation experiments were conducted at a power of 1.0 W~~mW~~ during 10 or 20 minutes.

*Uncaging of Poc-Minaprine assays monitored via LC-MS analysis.*

200 µL of 100 or 500 µM **Poc-Minaprine·HCl** solution in PBS (1:10 diluted in water) were added over 26 Pd@CLLCs and kept at 37 ºC for 24 hours or 48h. For NIR-stimulated uncaging experiment, see procedure above. At the time cap, 150 µL aliquots of solution were taken, filtered and submitted to LC-MS analysis using a gradient 5-25 MeCN-$H_2O$ 4 minutes. The concentration values were obtained by interpolating the experimental peak area values with a retention time of 2.35 minutes on a calibration curve. The used chromatographic method enables the simultaneous detection of Minaprine and **Poc-Minaprine** as sharp and well-separated peaks at 2.35 and 3.60 minutes, respectively. Experiments were performed in triplicate.

**Supplementary Table S3.** Uncaging of 100 and 500 µM Poc-Minaprine at different time caps:

| | 100 µM Poc-Minaprine | 500 µM Poc-Minaprine |
|---|---|---|
| **time (h)** | **[Minaprine] (µM)** | **[Minaprine] (µM)** |
| 24 | 6.2 ± 0.64 | 20.9 ± 2.7 |
| 48 | 9.2 ± 2.6 | 38.4 ± 7.5 |

*In vivo and ex vivo experiments.*

All procedures were approved by the UK Home Office and performed under PPLs PB5FC9BD2 and PP0330540. All experiments align to ARRIVE guidelines and those of the University of Edinburgh.

*Sox2*$^{CreERT2/+}$*; R26*$^{mTmG/+}$ mice were generated by breeding *Sox2*$^{CreERT2/+}$ *mice*[16] *with R26*$^{mTmG/mTmG}$ mice.[17] 8-12 week-old mice were administered with a single dose of 2.5 mg tamoxifen (Cambridge Bioscience) dissolved in sunflower oil (Sigma Aldrich), by oral gavage to induce recombination and labelling of SOX2+ cells. For *in vivo* experiments mice were administered a single dose of Minaprine dihydrochloire (10mg/kg; Merck M3157) or saline by intraperitoneal (I.P.) injection 24 hours later. After 48 hours mice were euthanized by a rising concentration of $CO_2$. For ex vivo experiments, mice were euthanized by a rising concentration of $CO_2$ 24 hours after the administration of tamoxifen. The sublingual glands (SLGs) were removed using sharp scissors and forceps. Fat and connective tissue was removed using forceps and the SLGs placed in a tube of phosphate-buffered saline (PBS; Invitrogen).

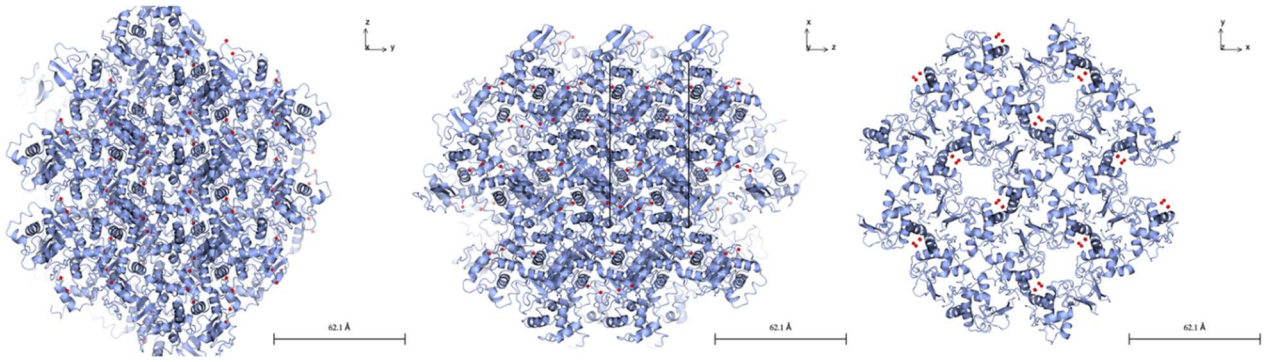
### *Organotypic slice culture*

The SLGs were embedded in 4% low-melting agarose (Merck) in a 35 mm petri dish. 200 μm slices of SLG were cut using a Vibratome (Leica) and slices transferred to pre-warmed RPMI media supplemented with 1% penicillin streptomycin solution.[18] Slices were cultured at 37 °C and 5% $CO_2$ on a 0.4 μm filter, above supplemented RPMI media. 100 nM Minaprine (Sigma Aldrich), 100 nM Poc-Minaprine or saline was added to the culture media. A single Pd@CLLC was placed in the centre of each SLG slice. Slices were cultured for 48 hours before analysis.

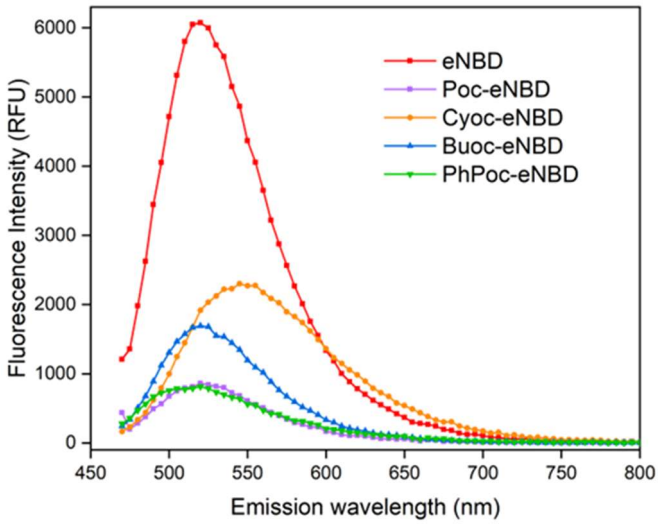
### *Immunofluorescent analysis*

Cell death was analysed using a NucView® 488 Caspase 3 kit (Biotium) following the manufacturer's instructions. Slices were imaged using the Opera Phenix High Content Imaging System (Revity). SLG slices were fixed in 4% paraformaldehyde (PFA; Thermo Fisher) for 20 minutes and washed in PBS. Slices were permeabilised with 0.1% Triton X (Sigma Aldrich) and incubated with Hoescht-33342 (1:1000; Sigma Aldrich) for 15 minutes to label nuclei. Slices were mounted using a SecureSeal spacer (Thermo Fisher) in Prolong Gold anti-fade mounting medium (Thermo Fisher). Images were acquired on a Leica SP8 confocal microscope. Fluorescent images were analyzed using National Institutes of Health ImageJ software and quantification undertaken using a segmentation pipeline, based on RACE and CellPose[19,20] and PickCells (https://www.software.ac.uk/blog/pickcells-and-exploratory-image-analysis-cell-biology). Normal distribution was assessed using the D'Agostino-Pearson omnibus test. Data were analysed for statistical significance using a student's *t*-test for pairwise comparison or one-way analysis of variance (ANOVA) with post-hoc testing performed using Tukey *Q* test (GraphPad Prism) for multi-parameter testing. For multiple testing, we used a false discovery rate of 0.05. All graphs show the mean + SD, as indicated in the figure legends.

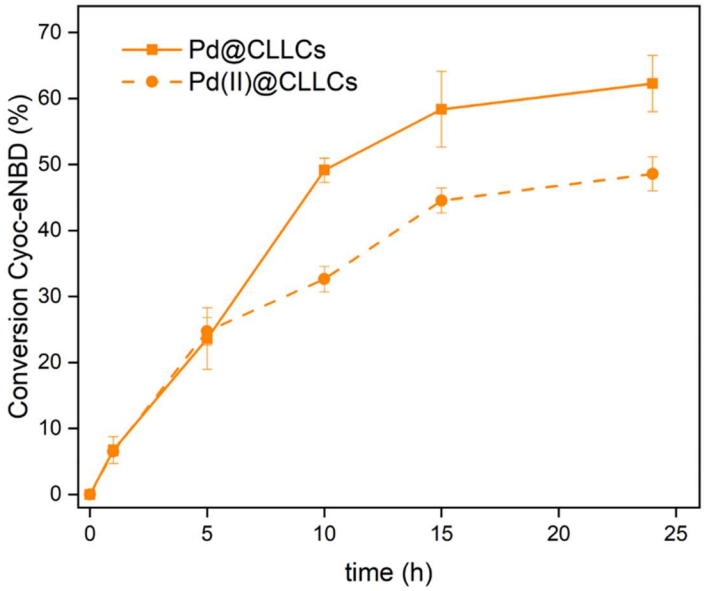
## 4. Supplementary Figures.



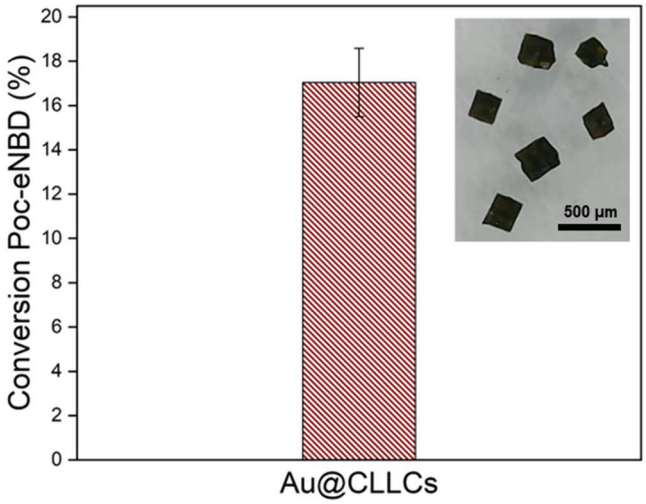
**Supplementary Figure S1. Ribbon representation of lysozyme crystal loaded with Pd(II) ions obtained from synchrotron source X-ray diffraction.** Pd(II) ions are represented in red and enlarged for visualization. The structures are projected from each crystallographic axis, from left to right, x, y and z.



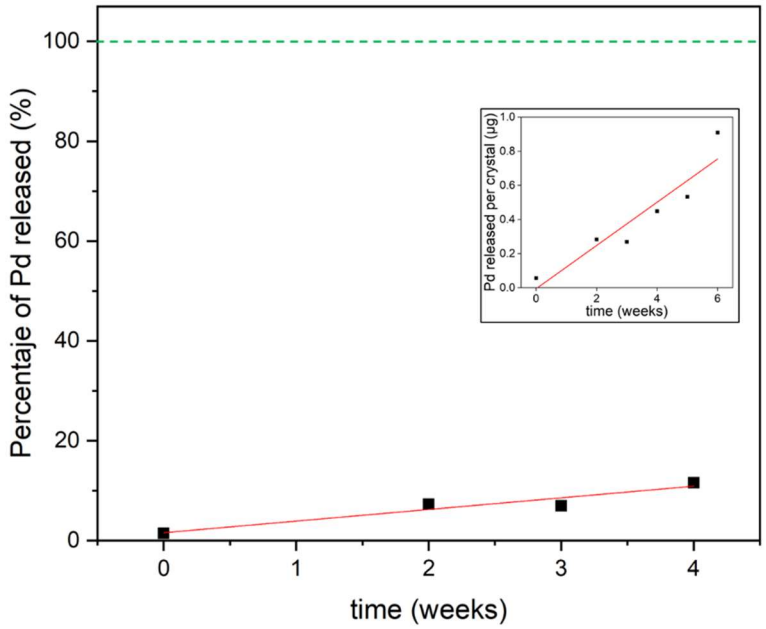

**Supplementary Figure S2. Fluorescence profile of all pro-eNBD prodyes employed in comparison with eNBD**. Excitation wavelength 440 nm, 1 cm path length.



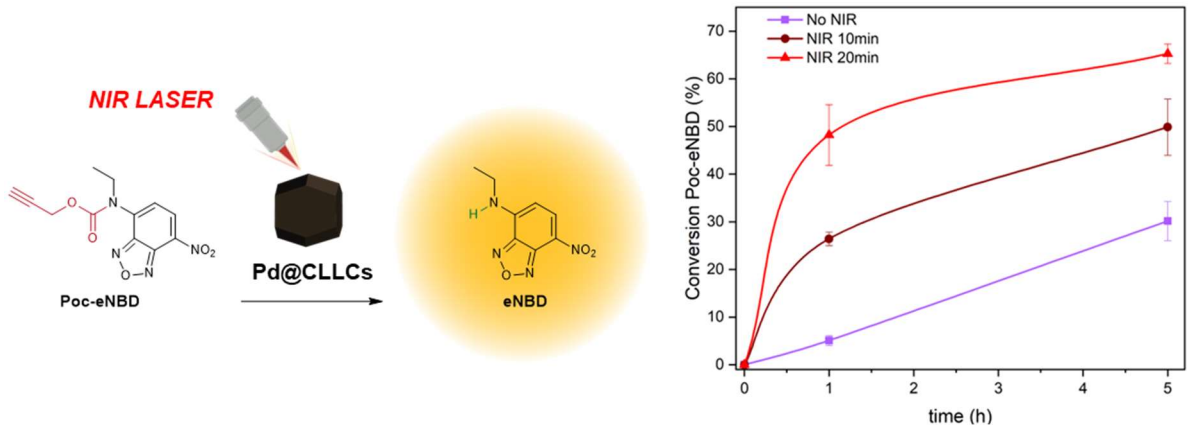

**Supplementary Figure S3.** Pd@CLLCs and Pd(II)@CLLCs-mediated uncaging of Cyoc-eNBD.



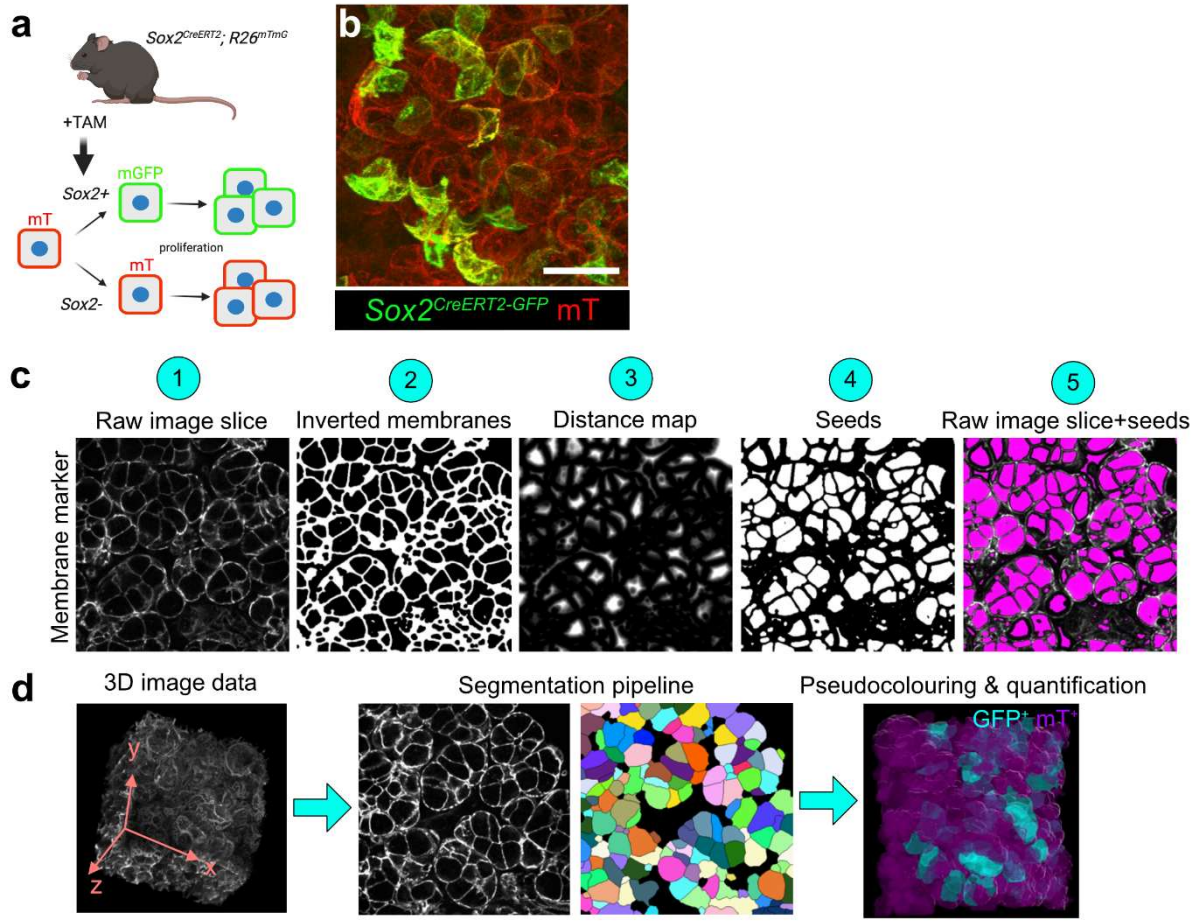

**Supplementary Figure S4.** Au@CLLCs-mediated uncaging of Poc-eNBD. Conversion after 24 h at 37 ºC of 20 µM of Poc-eNBD in PBS by 26 Au@CLLCs (insert: photograph of Au@CLLCs).



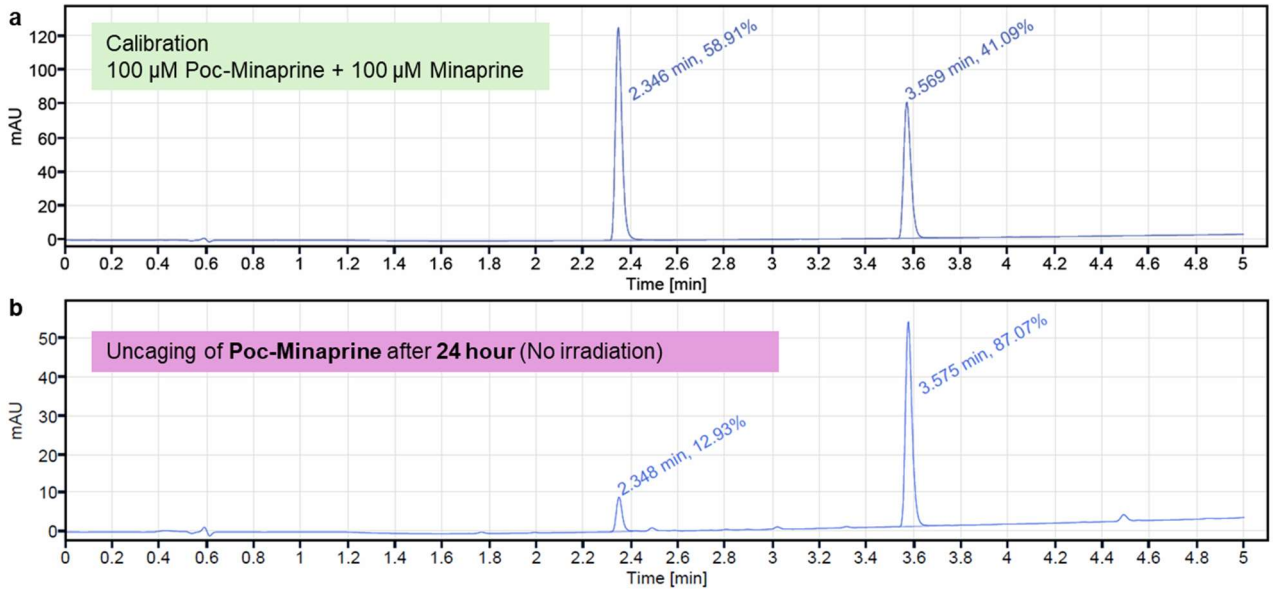

**Supplementary Figure S5.** Pd released from Pd@CLLCs under physiological conditions in function of time.



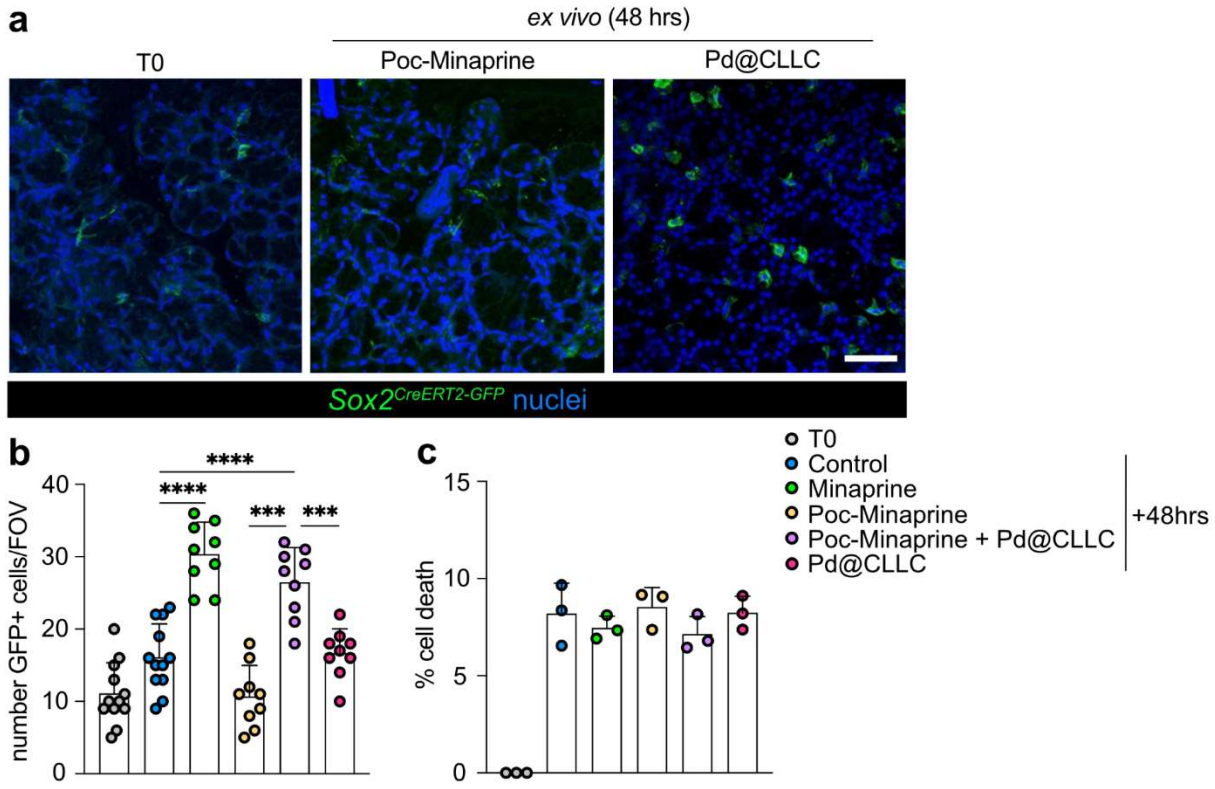

**Supplementary Figure S6.** Scheme of the NIR laser irradiation acceleration of the Pd@CLLCs-mediated uncaging of Poc-eNBD by incubation with 26 Pd@CLLCs in 250 µL of PBS when irradiated with NIR laser for 10 (vermilion) or 20 (red) minutes, and when they are not irradiated (violet).




**Supplementary Figure S7. a,** Description of the *Sox2CreERT2*; *R26mTmG* mice. **b,** Representative image of SMG from *Sox2CreERT2*; *R26mTmG* mice. mT = membrane-bound Tomato. Scale bar = 20mm. **c,** Schematic of the image analysis pipeline for cell segmentation, which involves sequential steps starting with a raw image file with a clear cell membrane marker; inverting black/white to highlight cell membranes; creating a distance map with increasing shading as the distance from a membrane increases; highlighting individual cells as "seeds" based on cell membrane analysis; overlay of the original raw image with the seeds to quality check the accuracy. **d,** Schematic of the image analysis pipeline for cell quantification, which involves taking 3D image data, progressing through the cell segmentation pipeline as shown in C, individual cells quantified as either GFP+ or GFP-, and pseudocoloured 3D images generated as quality control.




**Supplementary Figure S8.** LC-MS chromatograms of Pd@CLLCs-mediated uncaging of 100 µM of Poc-Minaprine·HCl in function of time and irradiation. **a,** Mixture of 100 µM of both Poc-Minaprine·HCl and Minaprine. **b,** Result of the incubation of Poc-Minaprine·HCl with Pd@CLLCs for 24 h at 37 ºC.




**Supplementary Figure S9. a,** Representative images of SMG slices from *Sox2CreERT2*; *R26mTmG* mice at time zero (T0), or treated with Poc-Minaprine alone, Poc-Minaprine + Pd@CLLC alone and analysed after 48 hours. Scale bar = 50mm. **b,** Enumeration of GFP+ cells from *Sox2CreERT2*; *R26mTmG* SMG slices at T0, or treated with saline, Minaprine, Poc-Minaprine alone, Poc-Minaprine + Pd@CLLC, or Pd@CLLC alone and analysed after 48 hours. *** $p<0.001$ **** $p<0.0001$. **c,** Enumeration of cell death in *Sox2CreERT2*; *R26mTmG* SMG slices at T0, or treated with saline, Minaprine, Poc-Minaprine alone, Poc-Minaprine + Pd@CLLC, or Pd@CLLC alone and analysed after 48 hours.

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