Supporting Information

Facile protein conjugation of platinum for light-activated cytotoxic payload release

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Materials and Methods
TBTU and formic acid were obtained from Fluka Chemicals and trifluoroacetic acid (TFA) from Fisher Scientific. trans,trans,trans-[Pt(N\textsubscript{3})\textsubscript{2}(Py)\textsubscript{2}(OH)\textsubscript{2}] (Pt\textsubscript{1}, Py = pyridine) was synthesised according to published procedures.\textsuperscript{1} Trastuzumab (Herceptin\textsuperscript{®}, Roche, 24.12 mg/mL in saline) was obtained from the Pharmacy Department at Guy’s and St. Thomas’ NHS Trust, London. All other chemicals were obtained from Sigma Aldrich and used without further purification. All H\textsubscript{2}O used in reactions was MilliQ ultrapure water to minimize unwanted metal contaminants. Silica gel used was technical grade, pore size 60 Å, 230-400 mesh, 40-63 μm particle size.

NMR spectra were recorded on a Bruker Avance III 500 MHz (for \textsuperscript{1}H) spectrometer and referenced to the residual solvent signal of the solvent. ESI-HRMS spectra in positive mode were recorded on a Bruker microTOF instrument in the range of 200–3000 m/z. Electronic absorption spectra were recorded on a Varian Cary 300 UV-vis spectrophotometer in a quartz cuvette at 298 K using neat solvent as reference. Platinum content was analysed on an ICP-MS 7500cx (Agilent).

LC-MS experiments were carried out on Bruker Amazon X ion trap instrument connected online with an Agilent 1260 HPLC. An Agilent ZORBAX Eclipse XDB-C18 column (250x4.6 mm, 5 μm) was used with 0.7 mL/min flow rate. The mobile phase contained 0.1% v/v formic acid in H\textsubscript{2}O (solvent A) and in CH\textsubscript{3}CN (solvent B). Gradient 1 included 5 min of equilibration at 10% B at the start of the run, then concentration of B increased from 10% (5 min) to 80% (at 30 min, maintained until 35 min) and subsequently decreased to 10% (at 40 min).

FTICR-MS experiments were carried out on a 12 T Bruker solariX instrument.

Synthesis and characterisation data
No problems were encountered during this work. However, heavy metal azides are known to be shock sensitive detonators therefore extra care was taken during handling.

All synthesis, purification and analysis, with the exception of the irradiation studies, were carried out in the dark with minimal light exposure.

Trans,trans,trans-[Pt(N\textsubscript{3})\textsubscript{2}(Py)\textsubscript{2}(OH)(OCO-(PEG)\textsubscript{2}-NH-Fmoc)] (Pt\textsubscript{2})
DIPEA (29.5 μL, 21.9 mg, 169.6 μmol) was added to a solution of TBTU (34.0 mg, 106 μmol) and Fmoc-NH-(PEG)\textsubscript{2}COOH (47.1 mg, 106 μmol) in DMF (1.2 mL). This was subsequently added to Pt\textsubscript{1} (50 mg, 106 μmol) in DMF (1.2 mL) and stirred at 45 °C for 24 h. The crude
product was then purified via column silica gel chromatography with increasing percentage of MeOH in DCM (0% - 5%). Purification was monitored using TLC using 10% DCM in MeOH as a mobile phase. Rf = 0.68. 26 mg of pure product were isolated (27% yield).

This complex was observed by DLS to form aggregates when in 5% DMSO in water, resulting in its UV-vis absorbance to increase over time as the aggregate dissolved (Figure S1). Similarly, 2 species were observed for this complex by NMR with a 80:20 ratio, identical coupling pattern, but slightly different chemical shift suggesting they belong to the same complex in different chemical environment. NMR peak assignments below are reported for the major species only.

**1H NMR (500 MHz, CD$_3$OD)** δH = 8.90 (dd, J = 5.5 Hz, J$^{195}$Pt−$^{1}$H = 26.5 Hz, 4H, H$_o$ in pyridine), 8.19 (t, J = 7.5 Hz, 2H, H$_p$ in pyridine), 7.80 (d, J = 7.5 Hz, 2H in FMOC), 7.74 (t, J = 6.5 Hz, 4H, H$_a$ in py), 7.65 (d, J = 7.5 Hz, 2H in FMOC), 7.39 (t, J = 7.5 Hz, 2H, in FMOC), 7.31 (d, J = 7.5 Hz, 2H in FMOC), 4.37 (d, J = 6.5 Hz, 2H, COOCH$_2$CH in FMOC), 4.20 (t, J = 6.5 Hz, 8H, OCH$_2$CH$_2$O in PEG$_2$), 3.57-3.63 (m, 8H, OCH$_2$CH$_2$O and OCH$_2$CH$_2$O in PEG$_2$), 3.26 (t, J = 5.4, 2H, OCH$_2$CH$_2$NH-FMOC), 2.57 (t, J = 6 Hz, 2H, Pt-OCOCH$_2$CH$_2$), 5.49 (DCM), 1.29, 0.9 (grease).

**13C NMR (126 MHz, CD$_3$OD)** δC = 177.8 (OCHO), 150.9 (C$, p$-pyridine), 144.0 (OCNH), 143.1 (C$p$-pyridine), 142.6 (aromatic CH in FMOC), 128.8 (C$m$-pyridine), 128.2, 127.4, 126.2, 121.2 (other aromatic CH in FMOC), 71.6, 71.3, 71.2, 70.9, 69.3 (CH$_2$O in PEG), 67.2 (NHCOOCH$_2$CH in FMOC), 55.9 (NHCOOCH$_2$CH in FMOC), 43.8 (OCH$_2$CH$_2$NH-FMOC), 41.8 (Pt-OCOCH$_2$), 38.6, 18.7 17.3 (DIPEA contamination).

HRMS: m/z [M+H]$^+$ calcd. 897.2644 found 897.2632, [M+Na]$^+$ calcd. 919.2464 found 919.2452.

**Trans,trans,trans-[Pt(N$_3$)$_2$(Py)$_2$(OH)(OCO-(PEG)$_2$-NH)$_2$] (Pt3)**

Piperidine (10 μL, 9.2 mg, 107 μmol) in DCM (1 mL) was added to Pt2 (24.1 mg, 27 μmol) and stirred at room temperature for 3 hrs. The crude mixture was diluted with DCM (4 mL), extracted 4x with water (5 mL). Water and piperidine were removed by rotary evaporation and the crude product redissolved in the minimum amount of water and purified via a C18 cartridge (SEP-PAK Plus Short, Waters) with increasing percentage of MeCN in H$_2$O (0% - 30%).

**1H NMR (500 MHz, D$_2$O)** δH = 8.80 (dd, J = 6 Hz, J$^{195}$Pt−$^{1}$H = 27 Hz, 4H, H$_o$) 8.28 (2H, t, J = 7 Hz, H$_p$) 7.81 (4H, t, J = 7 Hz, H$_m$) 3.70 (2H, t, J = 6 Hz, Pt-OCOCH$_2$CH$_2$) 3.64 (4H, broad
s, 1 PEG unit OCH$_2$CH$_2$O) 3.61 (2 H, t, J = 5 Hz, OCH$_2$CH$_2$NH$_2$) 3.58 (4 H, broad s, 1 PEG unit OCH$_2$CH$_2$O) 2.93 (2 H, t, J = 5 Hz, OCH$_2$CH$_2$NH$_2$) 2.64 ( 2 H, t, J = 6 Hz, Pt-OCOCH$_2$CH$_2$O).

$^{13}$C NMR (126 MHz, D$_2$O) $\delta_{C} = 178.1$ (CO) 149.2 (Co, pyridine) 142.7 (Cp, pyridine) 127.0 (Cm, pyridine) 69.6 (Pt-OCOCH$_2$CH$_2$O) 69.4, 69.3 (OCH$_2$CH$_2$O), (OCH$_2$CH$_2$O) 67.5 (OCH$_2$CH$_2$NH$_2$), 39.5 (OCH$_2$CH$_2$NH$_2$), 36.8 (Pt-OCOCH$_2$CH$_2$O).

HRMS: m/z [M+H]$^+$ calcd. 675.1962 found 675.1965.

**Trans,trans,trans-**[Pt(N$_3$)$_2$(Py)$_2$(OH)(OCO-(PEG)$_2$-NH-CSNH-Ph-NCS)] (Pt4)

Complex 3 (5 mg, 7.4 mmol) was dissolved in 1 mL DMF and 5 µL of DIPEA (29 mmol) was added. Para-phenylene diisothiocyanate (14 mg, 280 mmol) was separately dissolved in 500 µL DMF and added to the mixture that was stirred at room temperature for 40 min. DMF was removed by rotary evaporation at room temperature and the brownish residue dissolved in DCM. The product was purified by column chromatography (0-6% methanol in DCM). Purification was monitored by TLC using 10% DCM in MeOH as a mobile phase.

$^1$H NMR (500 MHz, acetone-d$_6$) $\delta_{H} = 8.99$ (dd, J = 6 Hz, J$^{195}$Pt-$^1$H$ = 27$ Hz, 4 H, H$_o$) 8.27 (2H, t, J = 7.2 Hz, H$_p$) 7.82 (4H, t, J = 7.2 Hz, H$_m$), 7.65 (t, J = 7.8 Hz, 2 H, NHCSNHC-CH$_2$), 7.28 (d, J = 9 Hz, 2 H, NHCSNHC-CH-CH$_2$), 3.72 ( 2 H, t, J = 6 Hz, Pt-OCOCH$_2$CH$_2$) 3.67 (2 H, m, OCH$_2$CH$_2$NH$_2$) 3.64-3.59 (10 H, OCH$_2$CH$_2$O and OCH$_2$CH$_2$O in PEG$_2$) 2.56 ( 2 H, t, J = 6 Hz, Pt-OCOCH$_2$H), 5.62 (DCM signal), 3.31, 3.30 (MeOH), 2.82, 2.78 (H$_2$O), 2.05 (acetone), 1.29, 0.9 (grease).

HRMS: m/z [M+H]$^+$ calcd. 866.17563 found 866.17562

**Conjugation to myoglobin**

700 µL of a 17 µM myoglobin solution in water was treated with a 1 M sodium carbonate solution to reach pH 8.5-9 (pH paper). A 1.7 mM solution of Pt4 in DMSO was then added in 5 µL aliquots to the myoglobin solution in a microcentrifuge tube, vortexing the tube after each addition to ensure full mixing. For the 5 mol. equiv. batch, 34 µL of Pt4 was added in total; for the 1 mol. equiv. batch only 7.5 µL was added. The reaction mixture was then vigorously mixed and left to incubate at room temperature for 4 h, followed by purification by size-exclusion chromatography using a PD10 column (GE Healthcare) preconditioned with 25 mM ammonium acetate.

S4
Size-exclusion HPLC
Size-exclusion HPLC analyses were carried out on an Agilent 1100 HPLC equipped with a DAD detector. An Agilent ZORBAX GF250 4-400 kDa size-exclusion column (250×4.6 mm, 4 μm) was utilised for the analysis, using 50 mM ammonium acetate in water as a mobile phase and a 1 mL/min flow rate. Detecting wavelength was set at 254 nm, reference wavelength at 360 nm.

Conjugation to trastuzumab
A PBS solution of trastuzumab (730 μL, 16.6 μM) was treated with 1 M sodium bicarbonate to reach pH 8.5, then 34 μL of a 1.7 mM DMSO solution of \( \text{Pt}_4 \) (5 mol. equiv.) was added in small aliquots and vortexing the solution between additions to ensure good mixing. After the final addition, the reaction mixture was vortexed and then left to incubate at room temperature for 2 h, followed by purification and buffer exchange to 100 mM ammonium acetate using 8 cycles of ultracentrifugation (Vivaspin MWCO 50,000).

Determination of platinum/protein ratios
Protein concentration for myo-Pt4 samples was determined by UV-vis spectroscopy using \( \varepsilon_{408} = 179,000 \text{ mol L}^{-1} \text{ cm}^{-1} \). Trastuzumab concentration in Trastuzumab-Pt4 samples was determined using a NanoDrop Lite spectrophotometer (Thermo Scientific) using settings for IgG. The concentration of platinum in samples of both myo-Pt4 and Trastuzumab-Pt4 was determined using ICP-MS. Samples were prepared in a 3.6% v/v HNO3 solution and analysed using a no-gas mode.

Dark stability and photodecomposition
Complexes Pt4 and its precursors were dissolved in 5% DMSO 95% water v/v; myo-Pt4 was in 25 mM ammonium acetate. For dark stability, the UV-Vis spectrum was monitored over time while keeping the solution in the dark. For photodecomposition studies, the UV-Vis spectrum was measured at the same time point following irradiation. For blue light irradiation (\( \lambda = 420 \text{ nm} \)), a LZC-ICH2 photoreactor (Luzchem Research Inc.) was used equipped with a temperature controller and 8 Luzchem LZC-420 lamps without light filtration. For green light irradiation (\( \lambda = 517 \text{ nm} \)), an LED source was used (BASETech model no. SP-GU10 230 V~50 Hz 1.3-2.1 W).
FT-ICR Mass spectrometry

A 12 T solariX instrument (Bruker Daltonik GmbH, Bremen, Germany) equipped with an infinity cell was used for these experiments. Nano-electrospray ionisation was performed in positive-ion mode using a home-built source. To produce emitters, 1.2-mm thin-walled glass capillaries (World Precision Instruments, Hitching, UK) were pulled in-house with a P97 Flaming/Brown type micropipette puller (Sutter Instrument Co., Novato, CA, USA) to obtain tips of ca. 1-μm orifice diameter. Data analysis was performed using Bruker Compass DataAnalysis 4.1, and peaks were assigned manually. For native MS, myoglobin was dissolved at a concentration of 10 μM in 25 mM aqueous ammonium acetate. MS measurements of myoglobin and trastuzumab under denaturing conditions were performed using 40 – 50% acetonitrile and 0.1 – 0.5% formic acid.
Table S1. ECD of \([\text{[Mb-Pt4]} + 15\text{H}]^{15+}\). Fragment assignment in ECD of \([\text{Mb} + 15\text{H}]^{15+}\) (first part) and \([\text{Mb-Pt4} + 15\text{H}]^{15+}\) (second part).

| Fragment | m/z (theory) | m/z (observed) | Error (ppm) |
|----------|--------------|----------------|-------------|
| [c5]+    | 447.2198     | 447.2199       | 0.2         |
| [c17]2+  | 957.4971     | 957.4973       | 0.2         |
| [c30]2+  | 1623.8308    | 1623.8308      | 0.0         |
| [c31]2+  | 1701.8813    | 1701.8815      | 0.1         |
| [c34]2+  | 1882.4814    | 1882.4803      | -0.6        |
| [c31]3+  | 1134.9233    | 1134.9235      | 0.1         |
| [c35]3+  | 1274.3305    | 1274.3322      | 1.3         |
| [c41]3+  | 1509.7734    | 1509.7740      | 0.4         |
| [c31]4+  | 851.4443     | 851.4451       | 0.9         |
| [c40]4+  | 1100.3212    | 1100.3223      | 0.9         |
| [c59]4+  | 1669.6078    | 1669.6079      | 0.1         |
| [c51]5+  | 1160.8138    | 1160.8145      | 0.6         |
| [c59]5+  | 1335.8877    | 1335.8876      | -0.1        |
| [c65]5+  | 1471.5640    | 1471.5648      | 0.6         |
| [c66]5+  | 1491.7735    | 1491.7717      | -1.2        |
| [c59]6+  | 1113.4076    | 1113.4080      | 0.3         |
| [c67]6+  | 1259.8239    | 1259.8237      | -0.1        |
| [c67]7+  | 1079.9929    | 1079.9938      | 0.8         |
| [c69]7+  | 1110.3004    | 1110.3005      | 0.1         |
| [c77]7+  | 1217.9397    | 1217.9399      | 0.1         |
| [c92]7+  | 1454.2104    | 1454.2110      | 0.4         |
| [c98]7+  | 1554.5522    | 1554.5516      | -0.4        |
| [c69]8+  | 971.6388     | 971.6399       | 1.1         |
| [c78]8+  | 1081.8350    | 1081.8353      | 0.2         |
| [c92]8+  | 1272.5600    | 1272.5609      | 0.7         |
| [c93]8+  | 1289.6924    | 1289.6911      | -1.0        |
| [c97]8+  | 1344.3472    | 1344.3469      | -0.2        |
| [c98]8+  | 1360.3591    | 1360.3593      | 0.1         |
| [c91]9+  | 1121.6062    | 1121.6066      | 0.4         |
| [c93]9+  | 1146.5052    | 1146.5064      | 1.1         |
| [c97]9+  | 1195.0872    | 1195.0878      | 0.5         |
| [c98]9+  | 1209.3200    | 1209.3198      | -0.1        |
| [c115]9+ | 1427.6682    | 1427.6696      | 1.0         |
| [c98]10+ | 1088.4887    | 1088.4895      | 0.7         |
| [z7]+    | 762.3913     | 762.3913       | 0.0         |
| [z8]+    | 925.4546     | 925.4544       | -0.2        |
| [z10]2+  | 562.7970     | 562.7968       | -0.4        |
| [z17]2+  | 977.5193     | 977.5202       | 0.9         |
| [z18]2+  | 1042.0406    | 1042.0404      | -0.2        |
| [z24]3+  | 900.1433     | 900.1441       | 0.8         |
| [z25]3+  | 919.1505     | 919.1514       | 1.1         |
| [z28]3+  | 1023.8580    | 1023.8585      | 0.5         |
| Fragment | m/z (theory) | m/z (observed) | Error (ppm) |
|----------|--------------|----------------|-------------|
| 447.2198 | 447.2199     | 0.1            |
| 957.4971 | 957.4973     | 0.2            |
| 1082.8896| 1082.8899    | 0.2            |
| 1134.9233| 1134.9230    | -0.3           |
| 1509.7734| 1509.7732    | -0.2           |
| Charge | Value 1   | Value 2   | Error  |
|--------|-----------|-----------|--------|
| [c31]4+| 851.4443  | 851.4450  | 0.8    |
| [c55]4+| 1565.8061 | 1565.8077 | 1.0    |
| [c43]5+| 961.2996  | 961.3004  | 0.8    |
| [c48]5+| 1092.3685 | 1092.3681 | -0.4   |
| [c59]5+| 1335.8877 | 1335.8868 | -0.7   |
| [c65]5+| 1471.5640 | 1471.5635 | -0.3   |
| [c66]5+| 1491.7735 | 1491.7724 | -0.8   |
| [c77]6+| 1420.7618 | 1420.7620 | 0.1    |
| [c93]7+| 1289.6924 | 1289.6910 | -1.1   |
| [c97]7+| 1344.3472 | 1344.3483 | 0.8    |
| [c97]9+| 1195.0872 | 1195.0876 | 0.3    |
| [c98]9+| 1209.3200 | 1209.3200 | 0.0    |
| [c98]10+| 1088.4887 | 1088.4894 | 0.6    |
| [z7]7+ | 762.3913  | 762.3912  | -0.1   |
| [z8]7+ | 925.4546  | 925.4547  | 0.1    |
| [z9]2+ | 527.2784  | 527.2784  | 0.0    |
| [z13]2+| 712.3711  | 712.3713  | 0.4    |
| [z14]2+| 769.3925  | 769.3933  | 1.0    |
| [z16]2+| 920.9773  | 920.9770  | -0.3   |
| [z17]2+| 977.5193  | 977.5198  | 0.5    |
| [z18]2+| 1042.0406 | 1042.0406 | 0.0    |
| [z18]3+| 695.0295  | 695.0300  | 0.8    |
| [z21]3+| 799.1015  | 799.1019  | 0.4    |
| [z24]3+| 900.1433  | 900.1441  | 0.9    |
| [z25]3+| 919.1505  | 919.1512  | 0.8    |
| [z27]3+| 985.5157  | 985.5155  | -0.2   |
| [z35]4+| 938.4671  | 938.4672  | 0.1    |
| [z36]4+| 970.4908  | 970.4912  | 0.4    |
| [z37]4+| 992.2488  | 992.2495  | 0.7    |
| [z38]4+| 1026.5136 | 1026.5141 | 0.6    |
| [z55]4+| 1517.7971 | 1517.7948 | -1.5   |
| [z60]4+| 1659.1305 | 1659.1300 | -0.3   |
| [z44]5+| 950.6956  | 950.6960  | 0.4    |
| [z49]5+| 1068.9465 | 1068.9460 | -0.4   |
| [z55]5+| 1214.4391 | 1214.4388 | -0.3   |
| [z55]6+| 1012.2005 | 1012.2013 | 0.8    |
| [z56]6+| 1033.5496 | 1033.5500 | 0.3    |
| [z57]6+| 1056.3928 | 1056.3929 | 0.1    |
| [z60]6+| 1106.4227 | 1106.4227 | 0.0    |
| [z75]6+| 1383.5659 | 1383.5645 | -1.0   |
| [z68]7+| 1073.4352 | 1073.4357 | 0.5    |
| [z70]7+| 1102.0180 | 1102.0188 | 0.7    |
| [z76]8+| 1053.9381 | 1053.9387 | 0.6    |
| [z94]9+| 1141.8486 | 1141.8489 | 0.3    |
| [z106]9+| 1292.3658 | 1292.3658 | 0.0    |
| [z94]10+| 1027.7644 | 1027.7647 | 0.2    |
| [c151+Pt4]13+ | 1356.4743 | 1356.4721 | -1.6 |
| [z152+Pt4]13+ | 1365.0914 | 1365.0915 | 0.1 |
**Figure S1.** UV-vis spectra of complex Pt2 (in 5% DMSO /95% water) at different time points over 2 h in the dark (Pt2 concentration: 50 μM, left) or upon irradiation with blue light (Pt2 concentration: 30 μM, right). Decrease of the LMCT attributed to Pt←N3 is evident upon irradiation. In contrast, an overall increase in absorbance is observed for the complex in the dark, attributed to the dissociation of aggregates over time.

**Figure S2.** UV-vis spectra of complex Pt3 (50 μM in 5% DMSO /95% water) at different time points over 2 h in the dark (left) or upon irradiation with blue light (right).
**Figure S3.** UV-vis spectra of complex Pt4 (50 μM in 5% DMSO /95% water) at different time points over 2 h in the dark (left) or upon irradiation with blue light (right).

**Figure S4.** Tandem MS of Pt4 showing its typical fragmentation pattern.
Figure S5. Pt4 size-exclusion HPLC (detection λ: 254 nm, reference: 360 nm, ΔA= A_{254} - A_{360}), inset shows the DAD spectrum for the main peak and corresponds to the UV-vis spectrum of Pt4.

Figure S6. UV-vis spectra of myoglobin (5.7 μM in 25 mM aqueous ammonium acetate) at different time points over 2 h upon irradiation with blue light, showing no photodecomposition.
Figure S7. (A) UV-vis spectra of the conjugate myo-Pt4 (25 mM aqueous ammonium acetate) over 5 h upon green light irradiation (3 μM myoglobin), showing reduction of the absorption band at 288 nm as well as the Soret band at 408 nm. (B) Percentage change in absorbance (288 nm - ● and 408 nm - ▲) at different time points in the dark (black lines), or upon irradiation (blue and green lines indicate 420 nm and 520 nm, respectively).

Figure S8. Size-exclusion HPLC of myo-Pt4 after 2h irradiation (detection λ: 254 nm, reference: 360 nm, ΔA= A_{254} - A_{360}). Photodecomposition of the conjugate is evident from the appearance of multiple peaks (compare to Fig. 2), but the mixture is too complicated for MS analysis.
Figure S9. FTICR-MS analysis of a 1:1 myo:Pt4 conjugation batch sample, showing the presence of free protein and 1:1 conjugate (A). Decomposition products of the 1:1 conjugate after 2h irradiation with blue light are shown in (B), with calculated and observed isotope distributions shown in insets. The 15+ charge state is shown as it is the most intense state present in the spectrum.
Figure S10. HPLC UV-vis chromatogram of Pt4 (detection wavelength 254 nm), gradient 1.

Figure S11. HPLC UV-vis chromatogram of Pt3 (detection wavelength 254 nm), gradient 1.

Figure S12. HPLC UV-vis chromatogram of Pt2 (detection wavelength 254 nm), gradient 1.
Figure S13. $^1$H NMR spectrum of complex Pt2 in CD$_3$OD.

Figure S14. $^{13}$C J-modulated spin-echo NMR spectrum of complex Pt2 in CD$_3$OD.
**Figure S15.** $^1$H NMR spectrum of complex Pt3 in D$_2$O.

**Figure S16.** $^{13}$C J-modulated spin-echo NMR spectrum of Pt3 in D$_2$O.
Figure S17. $^1$H NMR spectrum of complex Pt4 in acetone d-6.

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