In vivo metallophilic self-assembly of a light-activated anticancer drug

Supplementary Information

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Contents

1. Materials and methods

All reagents were purchased from commercial vendors. The reactants and solvents were used without further purification. All ^{1}H NMR, ^{13}C attached-proton-test NMR (^{13}C-APT NMR) were obtained on a Bruker DPX-300 spectrometers. Chemical shifts are indicated in ppm relative to the residual solvent peak. Electrospray ionization mass spectra (ESI-MS) were recorded by using an MSQ Plus Spectrometer positive ionization mode. The TEM experiments were carried via TEM JEOL 1010: 100 kV transmission electron microscope using Formvar/Carbon coated copper grid from Polysciences Inc. Uv-vis spectra were recorded on a Cary 50 spectrometer from Varian. The emission spectra and relative phosphorescence quantum yields were measured via an FLS900 Spectrometer from Edinburgh Instruments Ltd. The phosphorescence lifetime of the complexes in water was measured on a LifeSpec-II spectrometer from Edinburgh Instruments, using as excitation source a 375 nm pulsed diode laser. The singlet oxygen emission spectra were measured on a special custom-built setup which was described previously.(*1*) The DFT calculations were carried out using the Amsterdam Density Functional software (ADF2019) from SCM, the PBE0 functional, a triple zeta basis set (TZP), and COSMO to simulate the solvent effect in the water. Human cancer cell lines A549 (lung carcinoma), A431 (skin carcinoma) and A375 (malignant melanoma) were distributed by the European Collection of Cell Cultures (ECACC) and purchased from Sigma Aldrich. Dulbecco's Modified Eagle Medium (DMEM, with and without phenol red, without glutamine), Glutamine-S (GM; 200 mm), tris(hydroxylmethyl)aminomethane (Tris base), trichloroacetic acid (TCA), glacial acetic acid, and sulforhodamine B (SRB) were purchased from Sigma Aldrich. Opti-MEM Reduced Serum Media without phenol red was obtained from Gibco. The measurements of complexes on photocytotoxicity were performed according to the literature.(*2*) Annexin V/propidium iodide double staining assay was purchased from Bio-Connect BV. The FractionPREPTM Cell Fractionation kit was obtained from BioVision Incorporated.

Cyro-EM measurement. 6 μL of the sample ([**PdL**] = 25 μM) was applied to a freshly glow-discharged carbon 200 mesh Cu grid (Lacey carbon film, Electron Microscopy Sciences, Aurion, Wageningen, The Netherlands). Grids were blotted after a 10-sec wait for 3 sec at 99% humidity in a Vitrobot plunge-freezer (FEI VitrobotTM Mark III, Thermo Fisher Scientific). Cryo-EM images were collected on a Titan/Krios operating at 300 kV at a nominal magnification of 33000x or 81000x yielding a pixel size at the specimen of 3.5Å or 1.4 Å, respectively (NeCEN, Leiden University).

2D-monolayer photocytotoxicity experiments. For the cytotoxicity assay, Opti-MEM complete medium without phenol red was used, supplemented with 2.5% v/v fetal calf serum (FCS), 0.2% v/v penicillin/streptomycin (P/S), and 1% v/v Glutamine). Briefly, 100 µL Opti-MEM complete medium suspensions of A549 (5000 cells), A431 (8000 cells), or A375 (5000 cells) cells, were seeded into 96-wells plates and separated as dark or light groups, and incubated in the normoxic (21% O_2, 37 °C) or hypoxic (1% O_2, 37 °C) incubators. After 24 h, the cells were treated with **PdL** (100 µL) in a series of concentrations. At 48 h, the cell plates in the light group were irradiated with 520 nm green light with a dose of 13 J/cm^2 (normoxic-2D: 20 min, 10.92 mW/cm^2; hypoxic-2D: 32 min, 6.90 mW/cm^2), in normoxic (21% O_2) or hypoxic (1% O_2) conditions, while the dark group was kept in the dark. After irradiation, the cells were incubated in the dark for another 48 h. Then 100 µL of TCA fixation solutions (10% w/v) were added to the wells, and the plates were kept at 4 °C for 24 h. The photocytotoxicity of the complex was determined *via* the sulforhodamine (SRB) assay, and the normoxic or hypoxic half-maximal effective concentrations EC_{50} were obtained *via* Graphpad 8 using the dose-response two-parameter Hill-slope equation 1. Data are averages (n=3) with 95% confidence intervals (in µM) over three independent experiments.

$$100/(1 + 10^{log_{10}EC_{50} - X) \times Hill\ Slope}) \quad \textbf{Equation 1}$$

3D tumor spheroids viability assay. 100 µL Opti-MEM complete medium suspensions of A549 (500 cells), A431 (500 cells), or A375 (300 cells in normoxic conditions, 1000 cells in hypoxic conditions) cells were seeded into 96-well round-bottom Corning spheroid microplates and split as dark or light groups. Each plate was incubated for 3 days in normoxic or hypoxic conditions, to obtain 3D tumor spheroids. Then, the spheroids were treated with **PdL** (100 µL Opti-MEM complete medium) in a concentration series (0, 0.05, 0.25, 0.5, 1, 1.25, 2.5, 5, 12.5, 25). 24 h later, the plates of the light group were irradiated with 520 nm green light with a dose of 13 J/cm^2 (normoxia-3D spheroid condition: 32 min, 6.90 mW/cm^2; hypoxia-3D spheroid condition: 55 min, 3.99 mW/cm^2) and incubated for another 48 h. Then a CellTiter Glo 3D solution (50 µL/well) was added to each well to stain the 3D tumor spheroids. After 30 min shake on an IKA Vibrax shake at 500 rpm at room temperature, the luminescence in each well was measured by a Tecan Microplate Reader. Half-maximal effective concentrations (EC_{50}) for 3D tumor spheroids growth inhibition were calculated by fitting the CellTiter Glo3D dose-response curves using the same non-linear regression function as in 2D (Equation 1) as implemented in Graphpad Prism 8. Data are averages (n=3) with 95% confidence intervals (in µM) over three independent experiments.

Mode of cell death study using flow cytometry. 2×10^5 A375 cells were seeded in 12-well plates that were separated into dark and light groups, and incubated in normoxic condition. After 24 h, the cells were treated with **PdL** at a final concentration at 0.5 μM or 2 μM, and incubated for 24 h. Then, the light groups were irradiated with 520 nm green light with a dose of 13 J/cm^2 (20 min, 10.92 mW/cm^2), and incubated in normoxic condition for 2 h, 4 h or 24 h. Afterward, the cells in all groups were harvested with trypsin and stained with Annexin V/propidium iodide dyes for 15 min. The apoptosis status of cells was then determined via flow cytometry immediately. Parameter "GRN-B" (488 nm excitation, 525±30 nm emission) "RED-B" (488 nm excitation, 661±15 nm emission) were used for fluorescence measurements to match with the known excitation/emission wavelengths of Annexin V-FITC (494/518 nm) and propidium iodide (535/617 nm). All flow cytometry data were processed using FlowJo10.

Cellular uptake experiments. To measure simple cellular uptake, A375 cells (2×10^5) were seeded in 12-well plates and incubated for 48 h. Then the cells were treated with **PdL** (2 μM, 1 mL) for 2 h or 24 h. After that, the cells were washed by PBS for one time, and then harvested and centrifuged. After removing the supernatant, 0.5 mL of 65% HNO_3 was added to lyse the cell pellets with an overnight shake. For cellular uptake inhibition experiments, the cells were pretreated with different inhibitors for 1 h (NaN_3 (1 mg/mL), pitstop 2 (20 μM), dynasore (80 μM), nocodazole (40 μM), and wortmannin (4 μM)), or incubated at 4 °C for 30 min. Then, the cells were treated with **PdL** (5 μM) and incubated either in normoxic conditions (37 °C, 5% CO_2, 21% O_2, 100% humidity) or at 4 °C (in the air condition) for another 2 h. After that, the cells were harvested, centrifuged and lysed using the same method as in absence of inhibitor. Then, 9.5 mL of milli-Q water was added to the cell lysis solution to lower the HNO_3 concentration to 3.25% (v/v). The Pd content in the solution samples was measured *via* ICP-MS (NexION 2000, PerkinElmer).

***In vivo* tumor inhibition experiments.** Female BALB/c mice with 3 weeks old were originally purchased from Vital River Laboratory Animal Center (Beijing, China). The mice were kept under specific pathogen-free conditions with free access to standard food and water for 2 weeks, to let the mice weight around 20 g. This study was conducted following the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th edition, 2011). All protocols for animal studies conformed to the Guide for the Care and Use of Laboratory Animals. All animal experiments were performed under guidelines approved by the ethics committee of Peking University. The tumor model was established by inoculating 5×10^7 of A375 melanoma cells suspended in 100 μL of PBS at the right flank region of each mouse,

to obtain mouse A375 melanoma implant. 3 weeks later, the tumor volumes were around 100 mm^3. Tumor volume (V) can be calculated by formula $V = L/2 \times W^2$ after measuring the tumor length (L) and width (W).(*3*) The mice were then randomly divided into 4 groups (vehicle control, 520 nm light, **PdL**, **PdL** + 520 nm light groups, each group 4 mice). The injectable **PdL** solution were prepared by diluting the **PdL** stock DMSO solution (4.2 μM) to 420 μM using DMEM medium containing 10% v/v fetal bovine serum (FBS) and 1% v/v penicillin/ streptomycin (P/S). The mice were treated through tail intravenous injection with DMEM for vehicle control and 520 nm light groups, or **PdL** (2.1 μmol/kg, 420 μM, 100 μL DMEM medium (10% FBS), 0.9 mg/kg) for **PdL** dark and **PdL** + 520 nm light groups. After 12 h injection, 520 nm irradiation (100 mW/cm^2, 5 min) was then carried out twice, with an interval of 5 min for the light groups. Thus, the total light dose for each treatment was 100 mW/cm^2, 10 min, 60 J/cm^2. These treatment and irradiation steps were replicated at day 0, day 7 and day 14, respectively. On day 5, one mouse in each group was sacrificed and the tumor were taken up and fixed with paraformaldehyde (10 % v/v), then sectioned into slices and analyzed *via* H&E or TUNEL protocols, to evaluate the tumor cell damage and apoptosis conditions. The tumor volume and body weight of left mice (N=3) were measured and recorded and the average tumor volume and body weight were calculated over 20 days. At last, the mice were sacrificed, and the healthy organs were taken up, fixed with paraformaldehyde (10% v/v), then sectioned into slices and analyzed via H&E protocol, to determine their side effect after treatment.

Mice blood EM imaging experiments. The tumor-bearing mouse was treated with **PdL** (2.1 μmol/kg, 420 μM, 100 μL DMEM medium (10% FBS), 0.9 mg/kg) through intravenous tail injection. After 5 min, 1 mL of blood was taken up from the eye socket and diluted to 5 mL by PBS. After centrifugation (1500 rpm, 10 min), the supernatant was collected, and the left part was washed by PBS (5 mL) and centrifuged (1500 rpm, 10 min) again twice more, to obtain the supernatant PBS solution. These PBS solutions were then combined and centrifuged at a speed of 10000 rpm for 10 min. After removing the supernatant, 200 uL PBS were added and mixed well. Then the solutions were transferred to the TEM grids. For the preparation of TEM samples, a drop (15 μL) of the solution was added to the grids (formvar/carbon 200 Mesh, copper) and kept for 2 min, then the excess liquid on the grid was removed by filter paper, and dried for 2 h for TEM measurement. The TEM measurements were carried out in vacuum conditions (HITACHI H-7650).

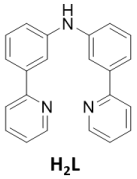
Mice tumor EM imaging experiments. One tumor-bearing mouse was treated with **PdL** (2.1 μmol/kg, 420 μM, 100 μL DMEM medium (10% FBS), 0.9 mg/kg) through intravenous tail

injection. After 12 h, the mouse was sacrificed, the tumor tissue was collected and then fixed by a biological TEM fixation solution (Wuhan Servicebio). After that, the tumor tissue was split into small pieces with volume around 1 mm^3, and fixed again using 1 % osmic acid PB solution for 2 h, following with dehydration by ethanol (v/v = 30 %, 50 %,70 %, 80 %, 95 %,100 %, each group 20 min) and acetone for two times (15 min). The prepared samples were then treated with acetone/epon-812 embedding medium in the ratio 1:1 for 2 h, 1:2 for 12 h, and pure epon-812 solution for another 5 h at 37 ˚C. After that, the tissue-containing embedding medium was filled in the embedding mold for 24 h at 37 ˚C, and another 60 °C for 48 h. The obtained tissue-containing resin were then sectioned into slices with thickness around 60-80 nm via ultramicrotome (Leica EM UC7), and moved to the copper grid (150 mesh). The obtained grids were stained by 2 % uranyl acetate ethanol solution for 8 min, and 2.6 % lead citrate solution for another 8 min. After that, the grids were dried at room temperature and observed using JEOL JEM2100 TEM (Japan).

Pd distribution determination on mice organs. The mice were treated with **PdL** (2.1 μmol/kg, 420 μM, 100 μL DMEM medium (10% FBS), 0.9 mg/kg) through intravenous tail injection. Then, the mice were sacrificed at 2 h, 6 h, 12 h, or 20 h, or 24 h, and their heart, liver, spleen, kidney, lung, and tumor, were taken. Then, around 1 g of each organs were lysed overnight in a mixture solution of 65 % HNO_3 (5 mL) and 30 % H_2O_2 (2 mL) at 100 °C. Afterward, each sample was evaporated and another 5 mL HNO_3 solution (2 %) was added. The Pd content in each organ or tumor was detected *via* ICP-OES (JY-Horiba ICP-OES Ultima 2).

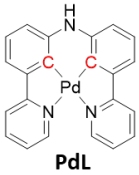
2. Synthesis and NMR characterization of ligands and metal complexes.

Synthesis of H_2L, (bis(3-(pyridin-2-yl)phenyl)amine)



A mixture of 2-(3-bromophenyl)pyridine (329 mg, 1.41 mmol), $Pd(dba)_2$ (81 mg, 0.14 mmol), racemic BINAP (106 mg, 0.17 mmol) and KO*t*-Bu (1574 mg, 14 mmol) was partially dissolved in dry toluene (28 mL) under N_2 atmosphere. The mixture was stirred for 10 min, then 3-(2-Pyridyl)aniline (230 mg, 1.35 mmol) was added, followed by heating the reaction mixture to 95 °C. After 3 days of stirring, the brown mixture was cooled down. Demi water (75.0 mL) was added and the mixture was stirred for 1 h. The H_2O layer was separated from the toluene layer. Extracted the H_2O layer with EtOAc (100 mL) for three times and combined the toluene and EtOAc layers, followed by rotary evaporation of the solvents. The crude product was purified by silica chromatography using pentane-EtOAc mixtures (2:1, R_f = 0.3) as eluent, to afford 290 mg of the target compound **H_2L**[1] (yield: 0.90 mmol, 67%). **ESI-MS** (cation): m/z calcd 324.2 ($C_{22}H_{17}N_3 + H^+$), found 324.7. **^{1}H NMR** (300 MHz, DMSO-d_6): δ 8.65 (dt, J = 4.7, 1.4 Hz, 2H), 8.49 (s, 1H), 7.97 – 7.81 (m, 6H), 7.53 (dt, J = 7.8, 1.3 Hz, 2H), 7.42 – 7.30 (m, 4H), 7.20 (dd, J = 7.8, 2.3 Hz, 2H). **^{13}C-APT NMR** (75 MHz, DMSO-d_6): δ 156.1, 149.5, 143.8, 139.7, 129.6, 122.6, 120.2, 120.2, 118.1, 117.4, 115.1.

Synthesis of PdL



A mixture of **H_2L** (90 mg, 0.28 mmol) and $Pd(OAc)_2$ (63 mg, 0.28 mmol) in a glacial acetic acid was refluxed for 24 h at 135 °C under N_2 atmosphere to give a yellowish green solution. Then the solvent was rotary evaporated. The crude product obtained was purified by silica chromatography using DCM/MeOH mixtures (v/v = 100:1.5, R_f =0.3) as eluent, to afford 67 mg of target complex **PdL** (yield: 0.15 mmol, 56%). **ESI-MS** (cation): m/z calcd 428.0379 ($C_{22}H_{15}N_3Pd + H^+$), found 428.0374. **^{1}H NMR** (300 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.94 (d, J = 5.4 Hz, 2H), 8.20 (d, J = 8.1 Hz, 2H), 8.10 – 8.00 (m, 2H), 7.49 (ddd, J = 7.1, 5.5, 1.3 Hz, 2H), 7.46 – 7.37 (m, 2H), 7.14 (t, J = 7.6 Hz, 2H), 7.00 (dd, J = 7.9, 1.1 Hz, 2H). **^{13}C** NMR (75 MHz, DMSO-d_6) δ 163.77, 148.75, 146.55, 139.56, 138.71, 137.87, 124.64, 122.74, 119.50, 115.14, 114.52. **Elemental analysis** calcd for **PdL**: C 61.77, H 3.53, N 9.82; found: C 61.93, H 3.64, N 9.60.

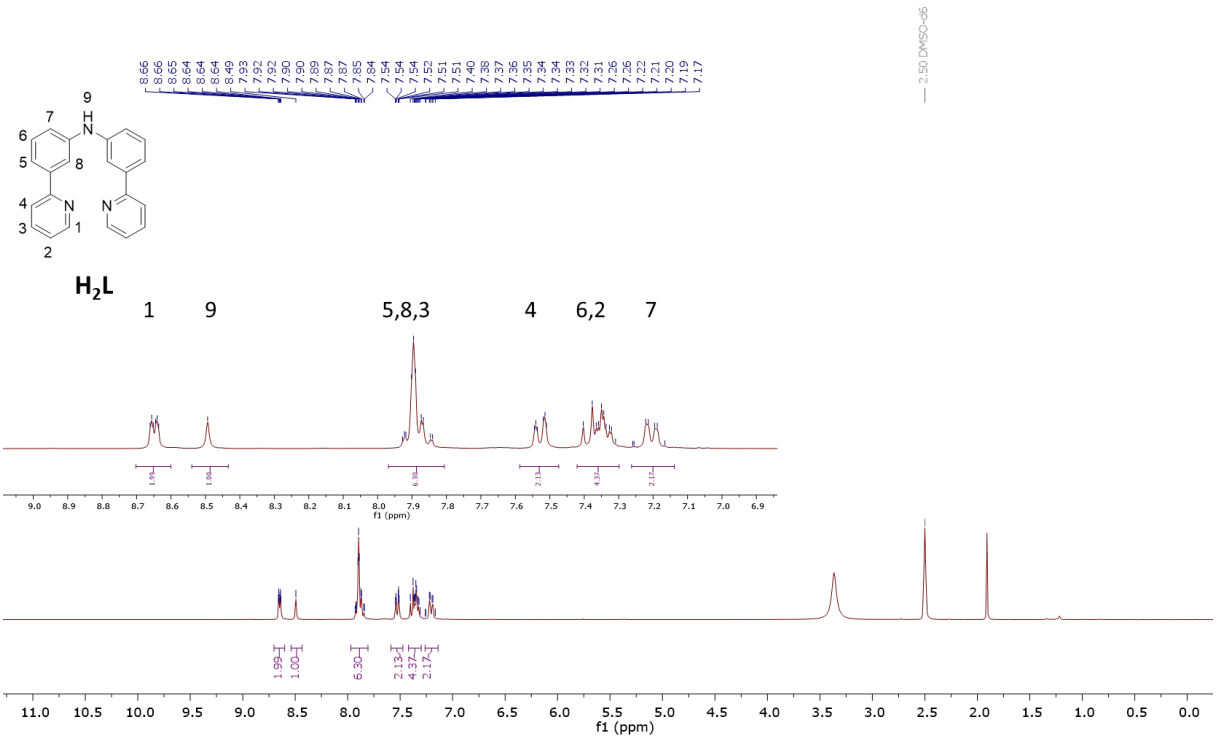


Figure S1. ^{1}H NMR of ligand **H_2L** in DMSO-d_6.

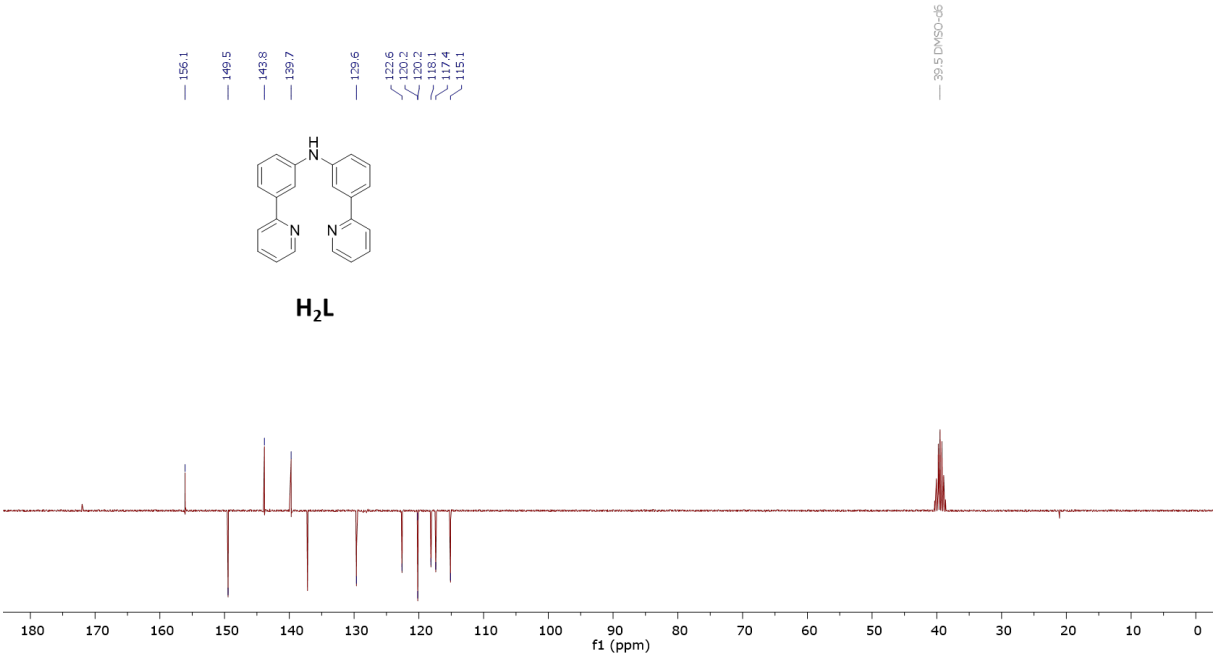


Figure S2. ^{13}C-APT NMR of ligand **H_2L** in DMSO-d_6.

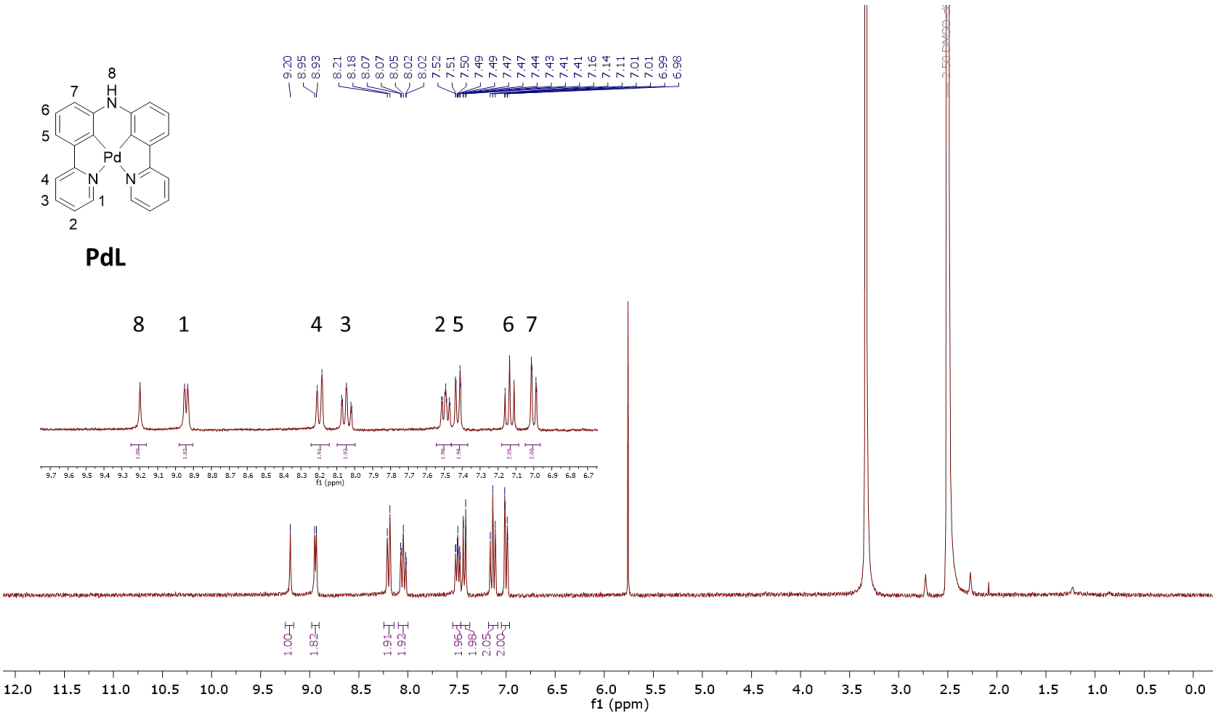


Figure S3. ^{1}H NMR of ligand **PdL** in DMSO-d_6.

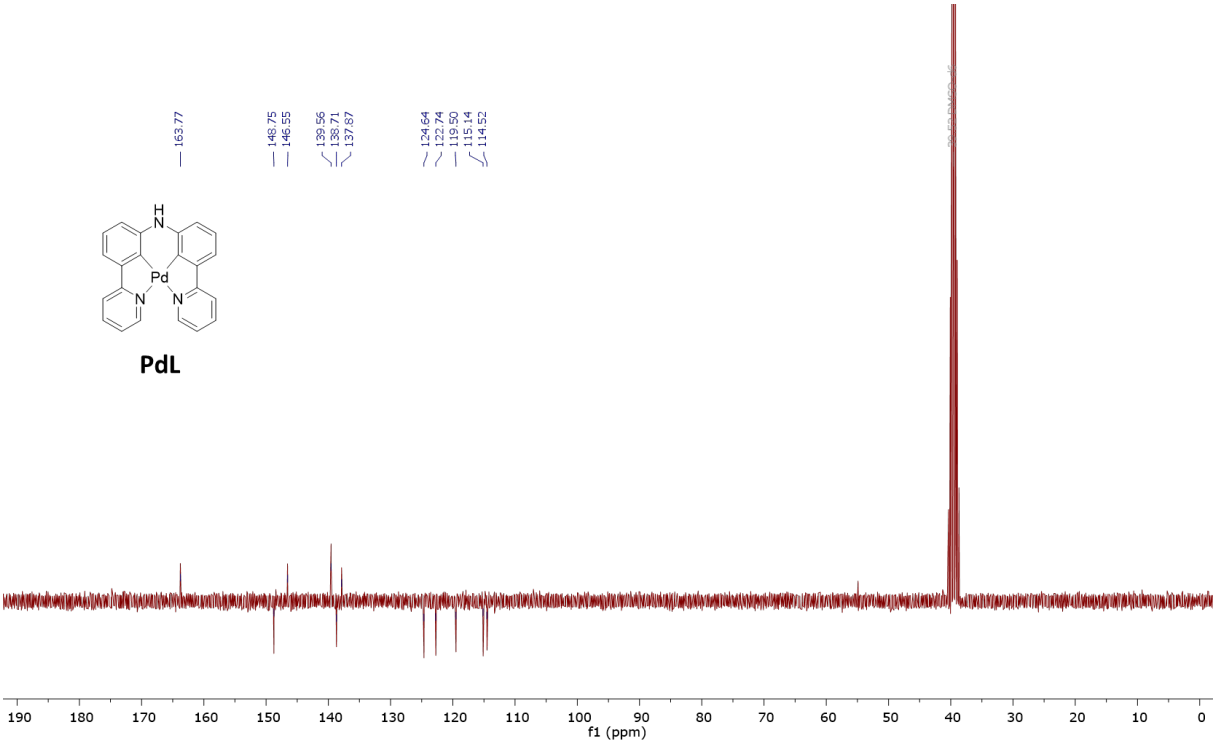


Figure S4. ^{13}C-APT NMR of ligand **H_2L** in DMSO-d_6.

3. Single crystal X-ray crystallography

All reflection intensities were measured at 110(2) K using a SuperNova diffractometer (equipped with Atlas detector) with Mo *K*α radiation (λ = 0.71073 Å) under the program CrysAlisPro (Version CrysAlisPro 1.171.39.29c, Rigaku OD, 2017). The same program was used to refine the cell dimensions and for data reduction. The structure was solved with the program SHELXS-2018/3 (Sheldrick, 2018) and was refined on F^2 with SHELXL-2018/3 (Sheldrick, 2018). Numerical absorption correction based on gaussian integration over a multifaceted crystal model was applied using CrysAlisPro. The temperature of the data collection was controlled using the system Cryojet (manufactured by Oxford Instruments). The H atoms were placed at calculated positions using the instruction AFIX 43 with isotropic displacement parameters having values 1.2 U_{eq} of the attached C or N atoms. The structure is ordered.

Table S1. Crystallographic data for **PdL**.

Complex	**PdL**
Crystal data	
Chemical formula	$C_{22}H_{15}N_3Pd$
M_r	427.77
Crystal system, space group	Monoclinic, $P2_1/n$
Temperature (K)	110
a, *b*, *c* (Å)	12.2301 (5), 10.3563 (4), 12.8772 (4)
β (°)	100.128 (4)
V (Å^3)	1605.59 (11)
Z	4
Radiation type	Mo *K*α
μ (mm^{-1})	1.17
Crystal size (mm)	0.09 × 0.08 × 0.03
Data collection	
Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Absorption correction	Gaussian *CrysAlis PRO* 1.171.39.29c (Rigaku Oxford Diffraction, 2017) Numerical absorption correction based on gaussian integration over a multifaceted crystal model Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{min}, T_{max}	0.880, 1.000
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	12313, 3695, 2920
R_{int}	0.048

$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.650
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.033, 0.072, 1.03
No. of reflections	3695
No. of parameters	235
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.85, -0.51

Table S2. Selected bond distances (Å) and angels (degree) in the crystal structure of **PdL**.

Distance (Å)		Angel (˚)	
Pd-N1	2.144(3)	C11-Pd1-C17	92.09(12)
Pd-C11	1.969(3)	C11-Pd1-N1	80.17(11)
Pd-C17	1.972(3)	C17-Pd1-N1	171.99(11)
Pd-N3	2.163(3)	C17-Pd1-N3	80.24(11)˚
Pd-Pd	3.518	N1-Pd1-N3	107.42(10)

4. DFT and TDDFT calculation of PdL

Table S3. Cartesian coordinates (Å) for the DFT-optimized geometries of a monomer of **PdL**.

PdL	X	Y	Z
Pd	1.076747	3.609677	3.371534
N	3.140108	4.118834	3.706555
C	1.054817	5.481678	2.764975
C	1.621249	0.85581	4.817111
H	2.551579	1.31205	5.118468
C	1.332168	-0.43798	5.200922
H	2.046516	-1.00031	5.786266
C	0.117442	-0.97782	4.816731
H	-0.14734	-1.99236	5.08659
C	-0.76518	-0.19756	4.096253
H	-1.72575	-0.59934	3.809388
C	-0.41644	1.103649	3.753914
C	-1.30531	2.038777	3.055654
C	-2.59136	1.677715	2.661317
H	-2.97374	0.679607	2.824682
C	-3.39939	2.623661	2.048635
H	-4.4022	2.359862	1.73592
C	-2.93495	3.90583	1.837127
H	-3.57553	4.640658	1.361305
C	-1.63807	4.27006	2.229642
C	-0.03802	6.172942	2.232752
C	0.072789	7.535195	1.917038
H	-0.78248	8.061274	1.506351
C	1.258108	8.210723	2.126733
H	1.324683	9.263149	1.880034
C	2.359763	7.548881	2.646401
H	3.281323	8.093896	2.799563
C	2.252524	6.195381	2.957406
C	3.390772	5.424361	3.466791
C	4.662668	5.949401	3.663834
H	4.855109	6.996713	3.483107
C	5.688128	5.124873	4.081877
H	6.681065	5.526579	4.238159
C	5.429942	3.780217	4.281078
H	6.204681	3.090087	4.585571
C	4.142078	3.326128	4.079398
H	3.91354	2.279347	4.207423
N	-1.25519	5.572542	1.987677
H	-1.95854	6.164324	1.571291
N	0.79039	1.604984	4.095986
C	-0.79879	3.334963	2.842834

Table S4. Cartesian coordinates (Å) for the DFT-optimized geometries of a dimer of **PdL**.

$[PdL]_2$	**x**	**y**	**z**
Pd	4.436988	5.081469	14.25353
C	6.544542	6.764228	12.59443
H	6.79188	5.819033	12.13876
C	7.21959	7.903731	12.21131
H	7.994754	7.843992	11.46009
C	6.851357	9.104762	12.79122
H	7.34533	10.02815	12.51444
C	5.83246	9.111816	13.72109
H	5.520469	10.0415	14.17454
C	5.202484	7.921936	14.06898
C	4.103019	7.830488	15.0292
C	3.589487	8.959894	15.66053
H	3.999071	9.943485	15.472
C	2.522495	8.821837	16.53268
H	2.108315	9.691329	17.02977
C	1.979351	7.575015	16.76749
H	1.141415	7.468026	17.44998
C	2.498001	6.437822	16.13414
C	3.575866	6.546967	15.25176
C	2.273287	3.964932	16.04457
C	1.554904	2.89771	16.59957
H	0.747429	3.104901	17.29549
C	1.864896	1.593532	16.27198
H	1.29858	0.779867	16.70986
C	2.893012	1.328131	15.38263
H	3.119178	0.302693	15.12116
C	3.605293	2.388649	14.82972
C	3.318567	3.726847	15.14849
C	4.675744	2.169536	13.85765
C	5.079573	0.913255	13.41876
H	4.596669	0.025855	13.80171
C	6.092142	0.801282	12.48883
H	6.410163	-0.17419	12.14122
C	6.682479	1.953854	12.00137
H	7.465049	1.925546	11.25588
C	6.223865	3.166436	12.47087
H	6.647926	4.081164	12.08876
N	5.573598	6.753752	13.50555
N	1.884841	5.234887	16.41994
H	1.141085	5.277616	17.09844
N	5.25981	3.288484	13.3814
Pd	3.264387	5.274825	11.09966

C	1.156634	3.592024	12.75844
H	0.90921	4.537217	13.21407
C	0.481498	2.452529	13.14141
H	-0.2938	2.512242	13.89249
C	0.849817	1.251503	12.56154
H	0.355785	0.328122	12.83822
C	1.868848	1.244464	11.63181
H	2.18088	0.314791	11.17837
C	2.498913	2.434346	11.28407
C	3.598491	2.525821	10.32398
C	4.112125	1.396434	9.692705
H	3.702569	0.412827	9.881208
C	5.179189	1.534534	8.820642
H	5.593421	0.665069	8.323551
C	5.722292	2.78138	8.585861
H	6.56017	2.888427	7.903312
C	5.203605	3.918541	9.219242
C	4.125639	3.809355	10.10149
C	5.427854	6.391486	9.308416
C	6.146168	7.458741	8.753392
H	6.953722	7.251583	8.057554
C	5.836049	8.762906	9.080899
H	6.402274	9.576601	8.642962
C	4.807934	9.028256	9.970274
H	4.581678	10.05369	10.23167
C	4.095786	7.967702	10.52328
C	4.382625	6.629512	10.20457
C	3.025437	8.18676	11.49548
C	2.621576	9.443017	11.9344
H	3.104326	10.33045	11.55132
C	1.609188	9.554927	12.86454
H	1.291149	10.53039	13.21218
C	1.01909	8.402318	13.3522
H	0.236717	8.430577	14.0979
C	1.47772	7.189758	12.88266
H	1.053868	6.274999	13.26492
N	2.127744	3.602511	11.8475
N	5.816562	5.121543	8.933283
H	6.560327	5.078845	8.254795
N	2.44157	7.067771	11.97189

Table S5. TDDFT singlet-singlet transitions calculation information of **PdL** in monomeric or dimeric state.

State	Energy (nm)	Energy (eV)	Oscillator strength (f)	Orbital transition contribution
Monomer	383.0	4.0697	0.1262	HOMO→LUMO 89.9%
	335.2	3.6989	0.3642	HOMO→LUMO+1 84.7%
	304.65	3.2369	0.7603	HOMO-1→LUMO 70.9%
Dimer	540.1232	2.2955	0.0043	HOMO→LUMO 100%
	501.6673	2.4714	0.0484	HOMO→LUMO 96%
	450.6614	2.7512	0.0168	HOMO→LUMO 47.3% HOMO-1→LUMO 36.9%
	400.7565	3.0938	0.0819	HOMO→LUMO+1 86.4%

5. Photophysical properties of PdL

Table S6. The photophysical properties of **PdL** in monomeric state.

Solvent	λ_{abs}, nm ($\varepsilon \times 10^{-3}$ M^{-1} cm^{-1})[a]	λ_{em} (nm)[a,b]	lifetime (ns)[a,d]	φ_p [c]	φ_Δ [c]
DMSO	343 (25.8), 405 (5.2), 481 (3.7)	564	0.406 ± 0.004	0.0008	0.09
THF	347 (22.5), 410 (4.3), 480 (2.9)	540	0.432±0.005		

[a] measurement were carried out in aerated DMSO or THF

[b] excitation and concentration: 419 nm, 100 μM.

[c] measurement was carried out in MeOD.

[d] excitation source : 340 nm. Monoexponential model: $y = y_0 + A_1 * \exp[-(x-x_0)\tau_1]$

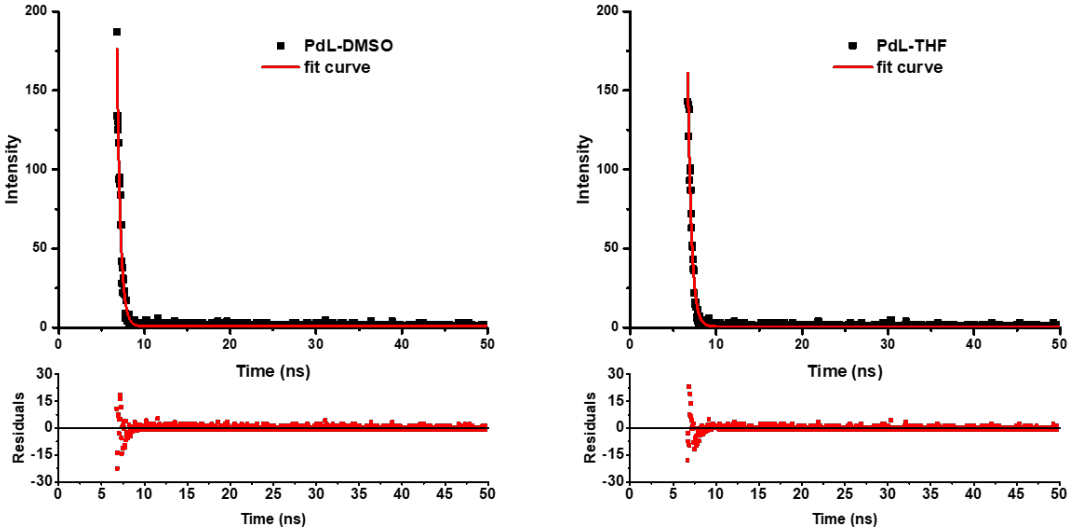


Figure S5. The phosphorescence lifetime spectra and fit curve of palladium complexes in aerated DMSO (left) and THF (right) at room temperature. Monoexponential fit equation: $y = y_0 + A_1 * \exp[-(x-x_0)\tau_1]$.

6. Self-assembly of PdL in solvent mixtures

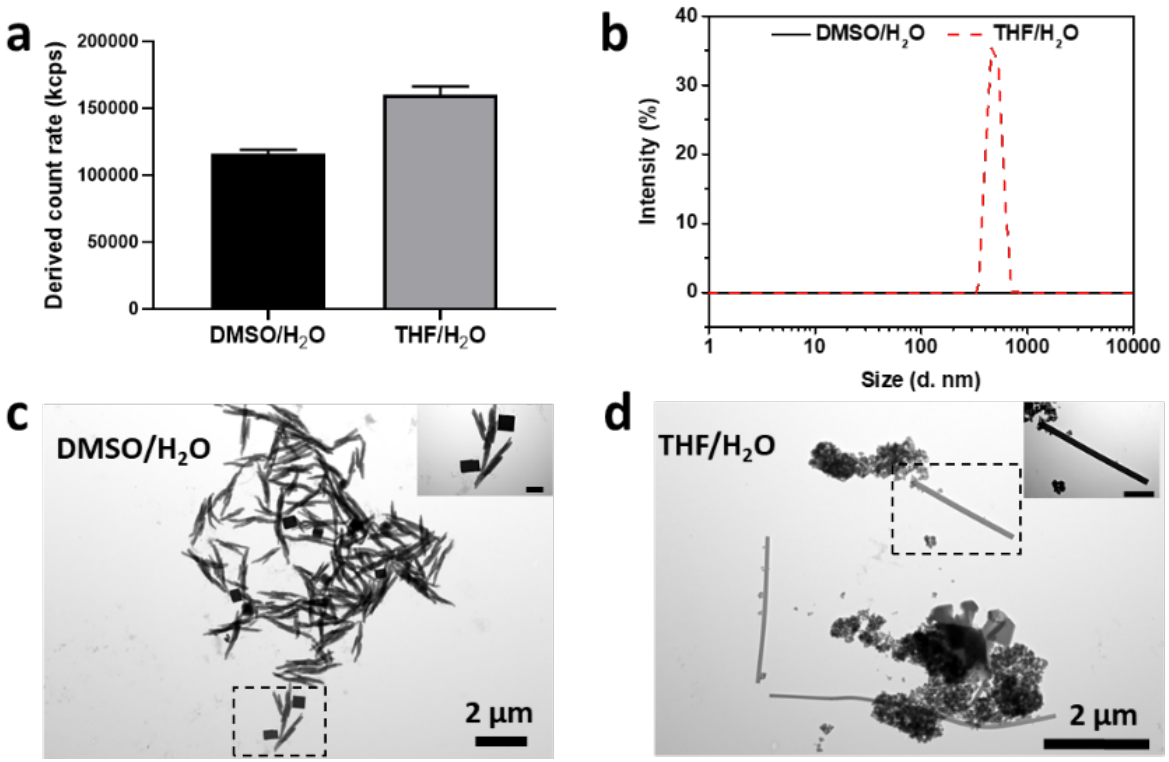


Figure S6. (a) DLS derived count rate in the DMSO/H_2O or THF/H_2O system of **PdL** (100 μM) after 30 min self-assembly; (b) Size distribution of the DLS analysis in the DMSO/H_2O or THF/H_2O system of **PdL** (100 μM) after 30 min self-assembly; TEM images of samples prepared from the DMSO/H_2O (c) or THF/H_2O (d) system of **PdL** (100 μM) after 30 min self-assembly. Inset picture scale bar: 500 nm.

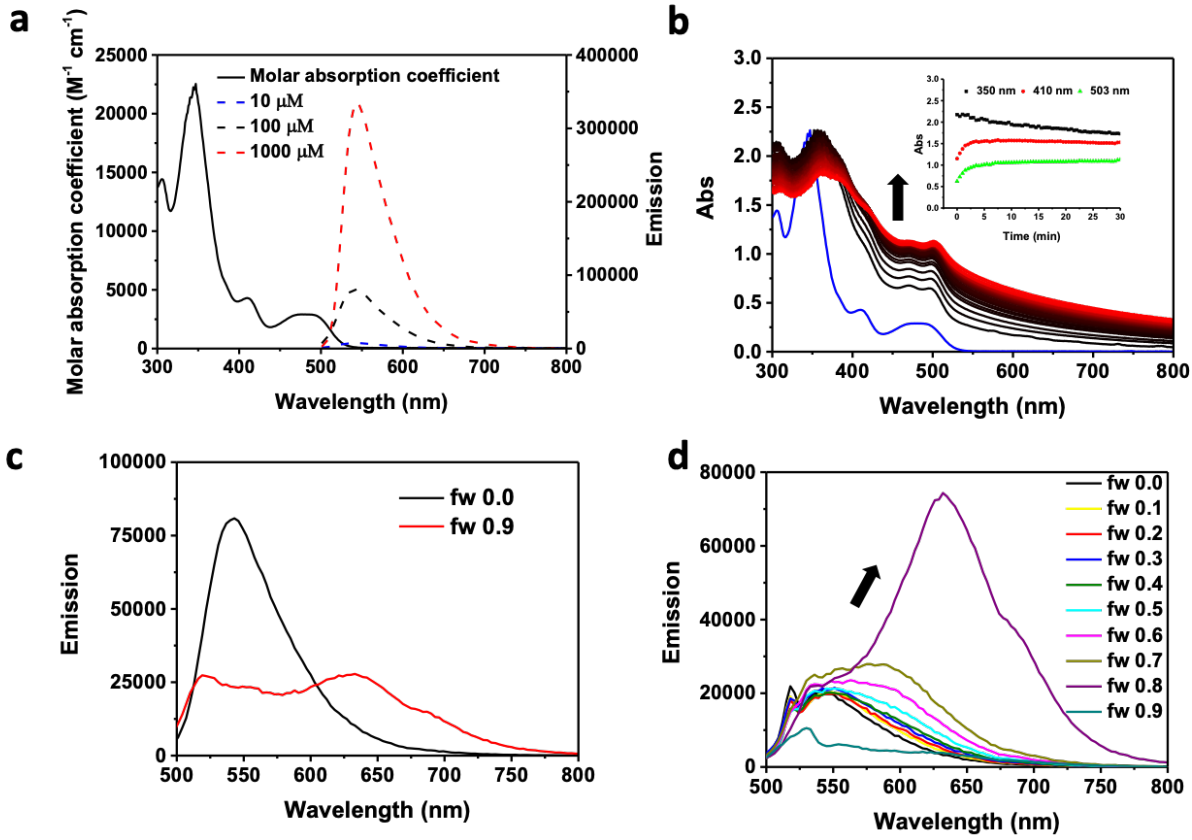


Figure S7. (a) The molar absorption coefficient (black solid line) and emission spectra of **PdL** in tetrahydrofuran (THF) solution at different concentrations (blue dash line 10 μM; black dash line 100 μM, red dash line 1000 μM). (b) Time evolution of the absorption spectra of H_2O/THF solution (100 μM, 9:1, v/v) of **PdL** at 298 K for 30 min (30 s interval, the color of spectra change from black (0 min) to red (30 min); the blue line is the absorbance spectra of **PdL** (100 μM) in pure THF). Inset: time evolution of the absorption at 350 nm (black square), 480 nm (red dot), 504 nm (green triangle) of the solution. (c) Emission spectra of **PdL** (100 μM) in pure THF (fw (V_{water}/V_{total}) = 0.0) and water/THF mixture (9:1, v/v, fw = 0.9); excitation 419 nm. (d) Emission spectra of **PdL** (20 μM) in different THF/water ratio (from v/v = 10/0 to 1/9, excitation 450 nm).

7. Photophysical properties of PdL in Opti-MEM complete medium

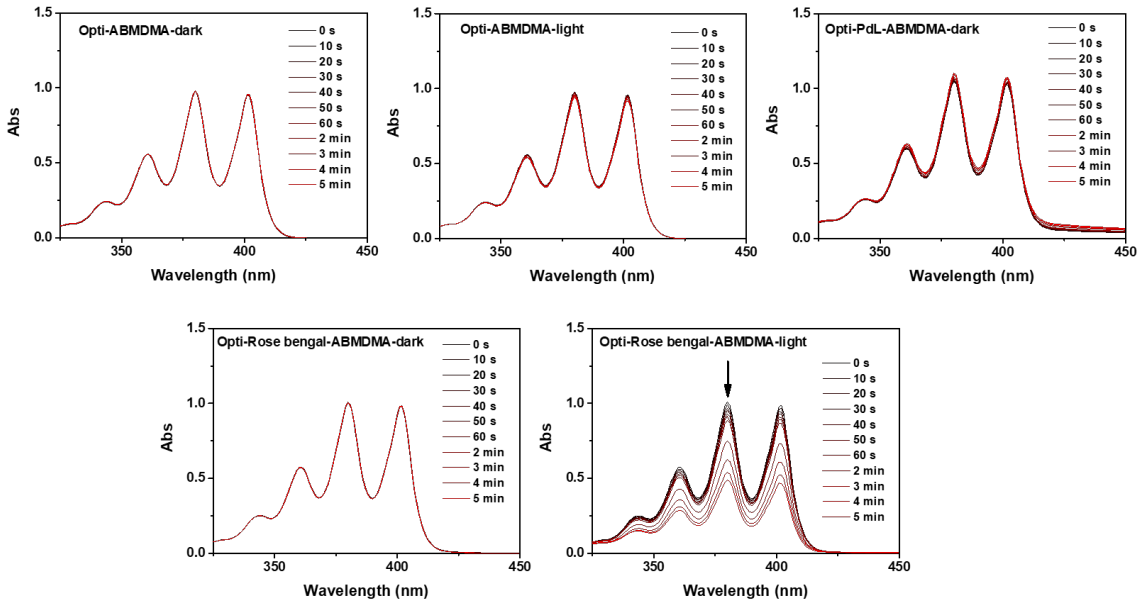


Figure S8. Time evolution of the absorption spectrum of an Opti-MEM complete solution of 9,10-anthracenediyl-bis(methylene)-dimalonic acid (ABMDMA, 100 µM) in the absence or presence of **PdL** (25 µM) or rose Bengal, under dark or green light irradiation (515 nm).

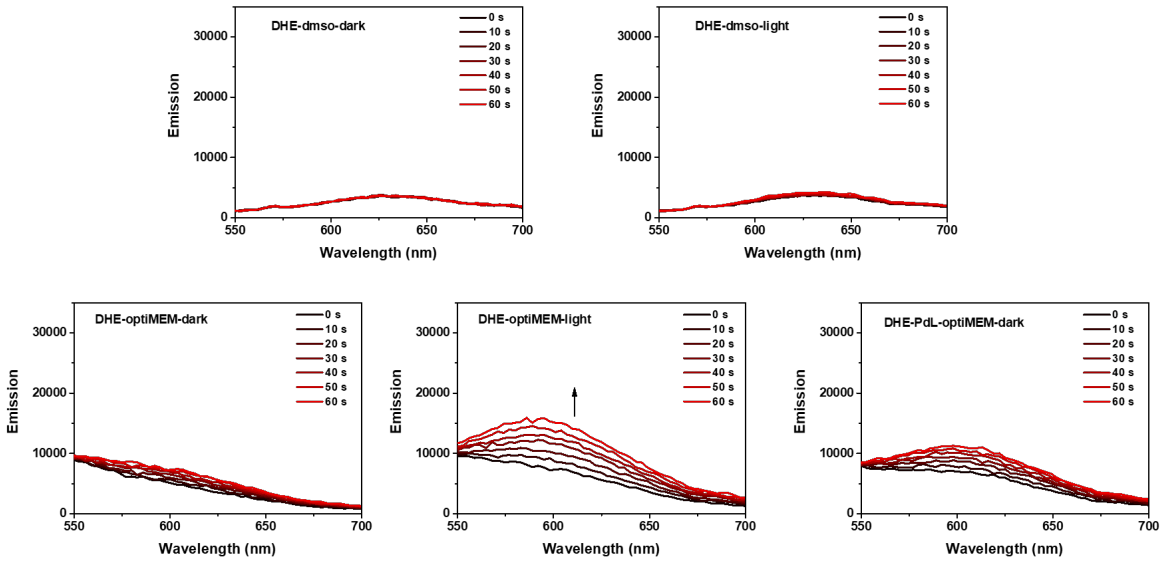


Figure S9. The emission spectra of dihydroethidium (DHE) solution (DMSO or Opti-MEM complete) in the absence or presence of **PdL** (25 µM) under green light irradiation (520 nm) or in the dark, over 60 s.

8. Photocytotoxicity of PdL and cell death mechanism determination in cancer cells

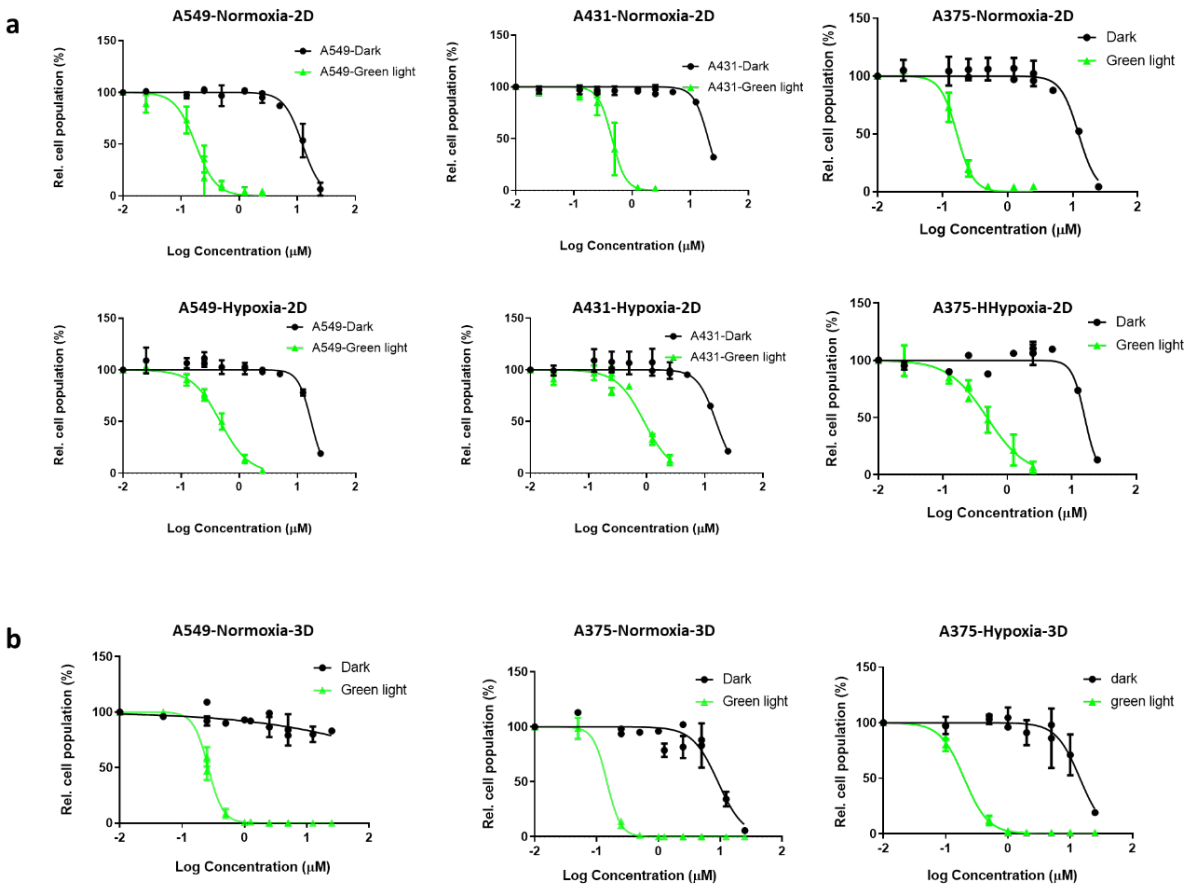


Figure S10. Dose-response curves for 2D-monolayer (a) or 3D-spheroid (b) for different human cancer cell lines incubated with **PdL**, either in the dark (black data points) or upon green light irradiation (green data points) under normoxic-2D (520 nm, 20 min, 10.92 mW/cm^2, 13 J/cm^2), hypoxic-2D (520 nm, 32 min, 6.90 mW/cm^2, 13 J/cm^2), normoxia-3D spheroid condition (520 nm, 32 min, 6.90 mW/cm^2, 13 J/cm^2), or hypoxia-3D spheroid condition (520 nm, 55 min, 3.99 mW/cm^2, 13 J/cm^2).

Table S7. Half-maximal effective concentration (EC_{50} in µM) of **PdL** for A549, A5431 and A375 cancer cells in normoxic, hypoxic or 3D-normoxic and 3D-hypoxic spheroids conditions under dark or green light irradiation. 95% confidence interval (CI in µM) and photoindex (PI = $EC_{50,\,dark}/EC_{50,\,light}$) are also indicated.

Cell line	EC_{50} Values (µM)							
	Condition	2D Normoxic		2D Hypoxic		3D Normoxic		3D Hypoxic
A549	dark	12	+1.3, -1.3	17	+1.5, -1.3	>25		
	light	0.18	+0.02,-0.02	0.47	+0.04, -0.03	0.26	+0.01, -0.01	N.D
	PI	68		37		>96		
A431	dark	20	+1.1, -1.1	16	+1.7, -1.6			
	light	0.45	+0.06, -0.05	0.90	+0.1, -0.1	N.D		N.D
	PI	45		17				
A375	dark	12	+1.3, -1.4	16	+1.6, -1.4	9.2	+2.1, -1.8	14 +4.9, -2.7
	light	0.17	+0.01, -0.01	0.49	+0.09, -0.07	0.17	+0.01, -0.02	0.20 +0.01, -0.01
	PI	72		32		54		72

Irradiation condition: normoxic 520 nm, 20 min, 10.9 mW/cm^2, 13 J/cm^2 ; hypoxic 520 nm, 30 min, 7.22 mW/cm^2, 13 J/cm^2; 3D-normoxic 520 nm, 32 min, 6.90 mW/cm^2, 13 J/cm^2; 3D-hypoxic 520 nm, 55 min, 3.99 mW/cm^2, 13.2 J/cm^2. Data are averages (n=3) with 95% confidence intervals (in µM) over three independent experiments. N.D means not determined.

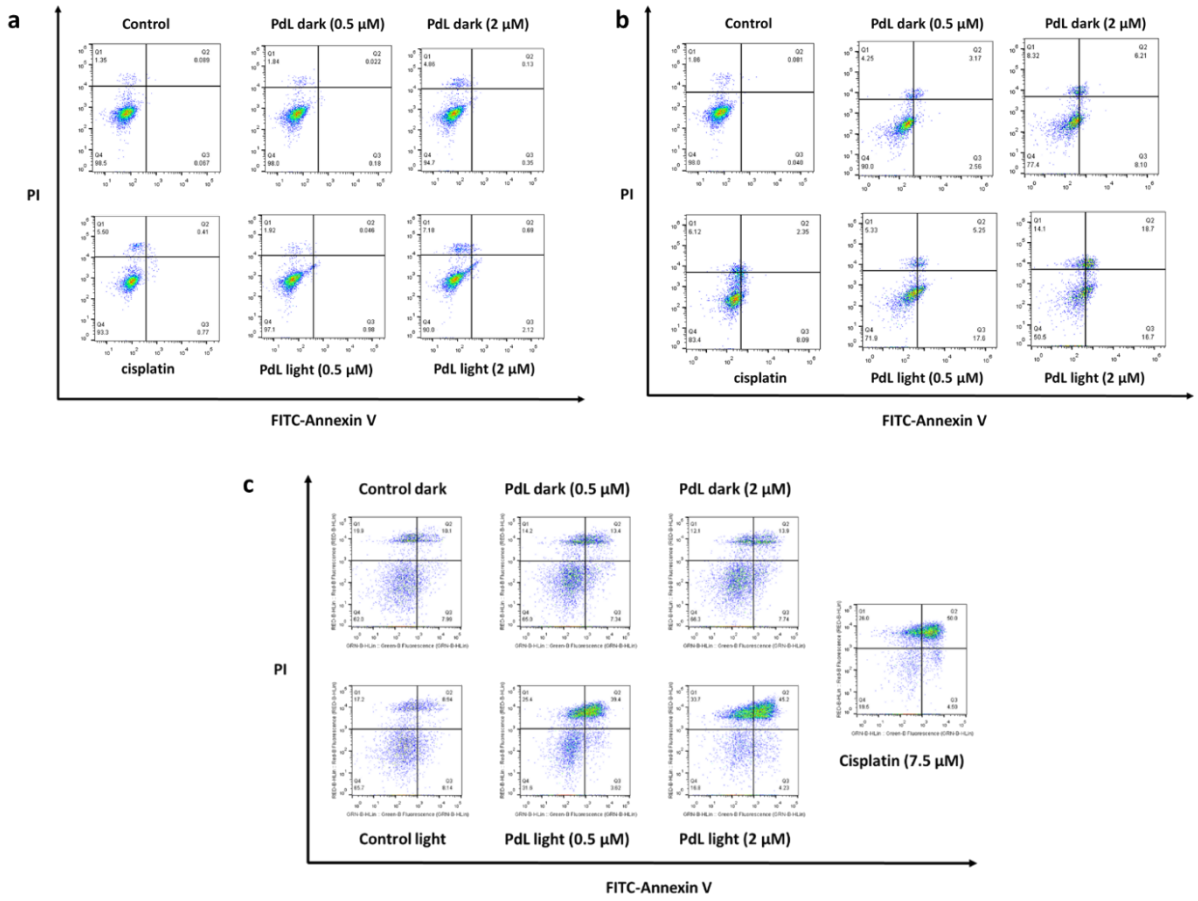


Figure S11. Annexin V/Propidium iodide double staining FACS data for A375 cells after treatment with cisplatin (7.5 µM) or **PdL** (0.5 µM or 2 µM) in the dark or upon green light irradiation (normoxic 520 nm, 20 min, 10.9 mW/cm^2, 13 J/cm^2) after 2 (a), 4 (b) and 24 h (c).

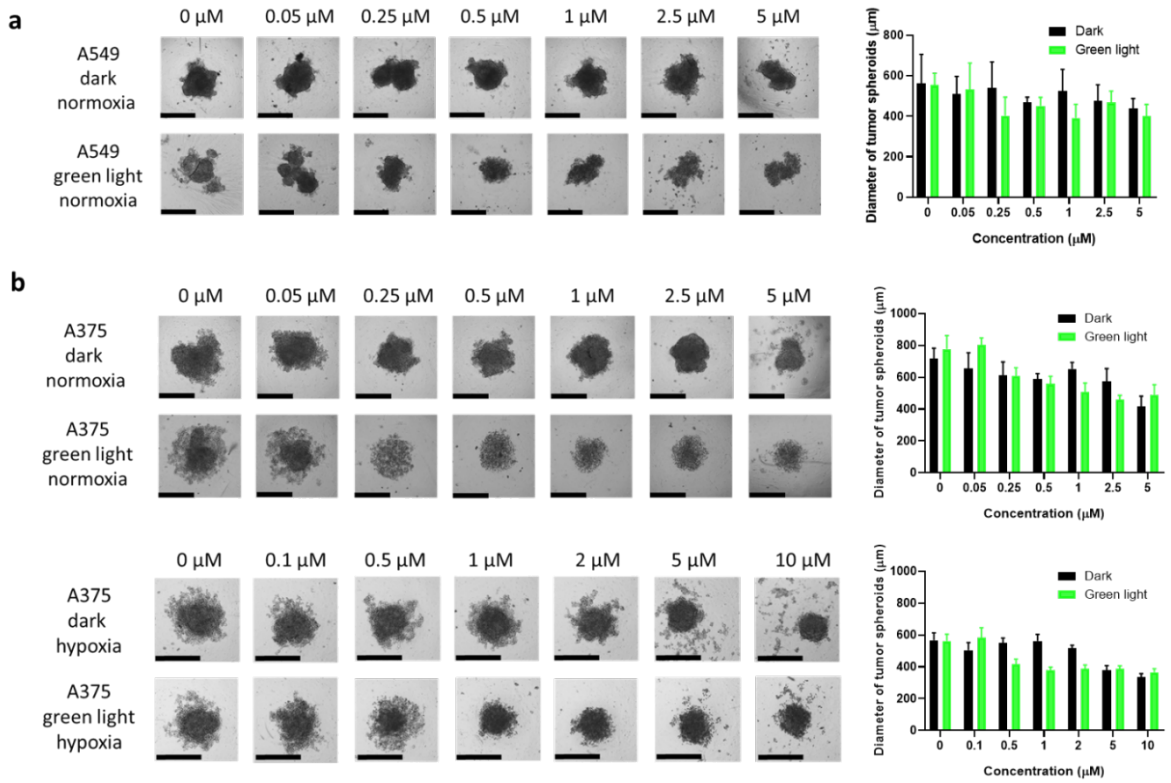


Figure S12. Bright filed images (left) and diameter (right, µm) for A549 (a) and A375 (b) 3D tumor spheroids kept in the dark (black bars) or irradiated with green light (green bars, 520 nm, 13 J/cm^2). Scar bar 500 µm.

9. Tumor xenografts experiments

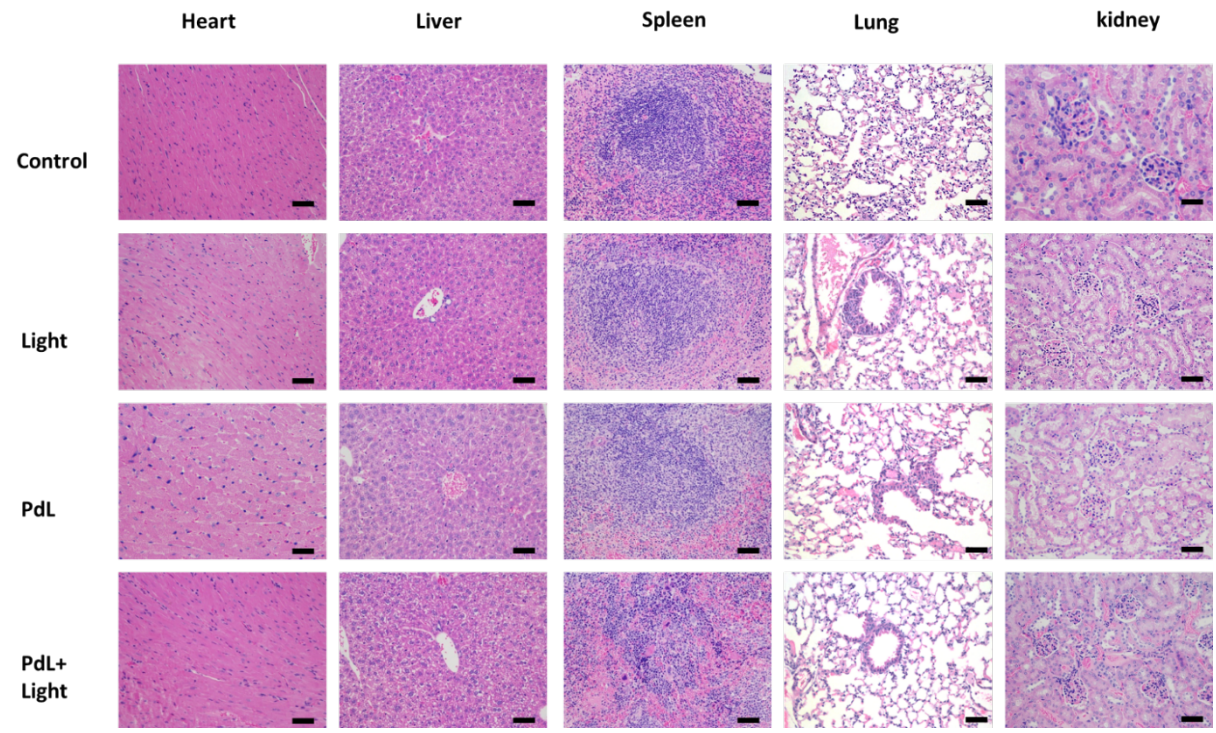


Figure S13. The H&E staining of different mice organs after treatment with vehicle control or **PdL**, and either without or with green light irradiation (100 mW/cm^2, 10 min, 60 J/cm^2). Scale bar 200 μm.

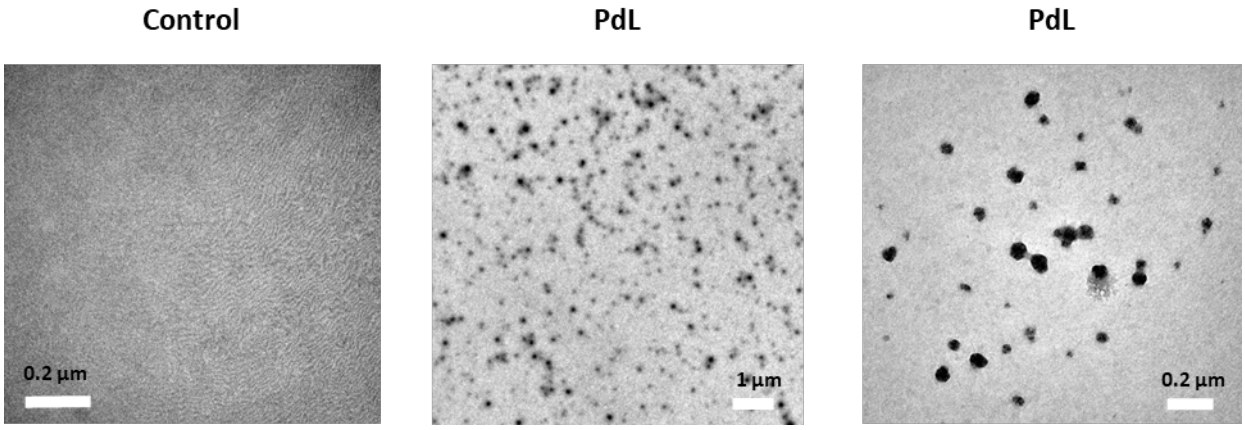


Figure S14. EM images showing the morphology of nanoparticles found in the blood of mice 5 min after intravenous tail injection of **PdL** (middle and right images), or in an untreated control mice (left image). Injection dose: 2.1 μmol/kg, 420 μM, 100 μL DMEM medium (10% FBS), 0.9 mg/kg.

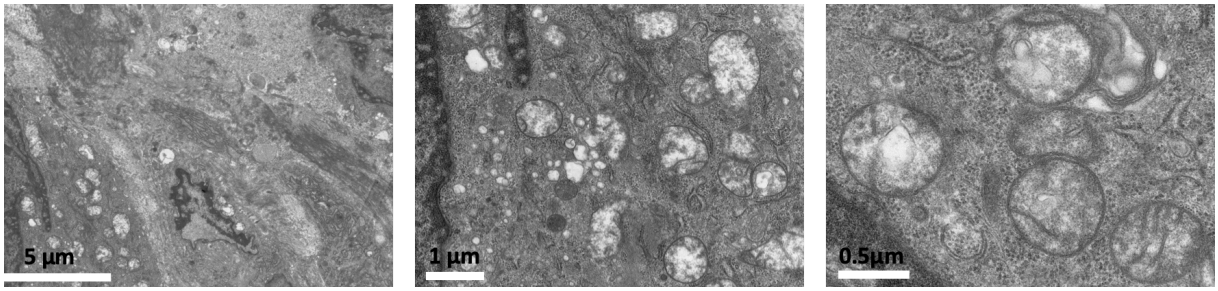


Figure S15. EM images at a different magnification of slices of A375 tumor xenografts without the treatment of **PdL**.

10. References

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2. X. Q. Zhou *et al.*, The self-assembly of a cyclometalated palladium photosensitizer into proteins-stabilized nanorods triggered drug uptake in vitro and in vivo. *J. Am. Chem. Soc.* **142**, 10383–10399 (2020).
3. W. Sun *et al.*, Biodegradable Drug-Loaded Hydroxyapatite Nanotherapeutic Agent for Targeted Drug Release in Tumors. *ACS Appl. Mater. Interfaces* **10**, 7832-7840 (2018).

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