The self-assembly of a cyclometalated palladium photosensitizer into proteins-stabilized nanorods triggered drug uptake in vitro and in vivo

Xue-Quan Zhou\textsuperscript{a}, Ming Xiao\textsuperscript{b}, Ramu Vadde\textsuperscript{a}, Jonathan Hilgendorf\textsuperscript{b}, Xuezhao Li\textsuperscript{b}, Panagiota Papadopoulou\textsuperscript{a}, Maxime A. Siegler\textsuperscript{c}, Alexander Kros\textsuperscript{a}, Wen Sun\textsuperscript{b,\ast}, and Sylvestre Bonnet\textsuperscript{a,\ast}

\textsuperscript{a} Leiden Institute of Chemistry, Universiteit Leiden, Einsteinweg 55 2333 CC, Leiden, Netherlands.
\textsuperscript{b} State Key Laboratory of Fine Chemicals, Dalian University of Technology, 2 Linggong Road, Dalian 116024, China
\textsuperscript{c} Department of Chemistry, Johns Hopkins University, Maryland 21218, Baltimore, USA

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1. General information

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 or DMX-400 spectrometers. Chemical shifts are indicated in ppm relative to the residual solvent peak. Electrospay ionization mass spectra (ESI-MS) were recorded by using a MSQ Plus Spectrometer positive ionization mode. High-resolution mass spectra (HRMS) of two palladium complexes were recorded on Waters XEVO-G2 XSQ-TOF mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.0 kV, desolvation gas flow 900 L/hr, temperature 250 °C) with resolution R = 22000 (mass range m/z = 50-2000) and 200 pg/uL Leu-enkephalin (m/z = 556.2771) as a “lock mass”. 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 a 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 (ADF2017) from SCM, the PBE0 functional, a triple zeta basis set (TZP) for all atoms including Pd, scalar relativistic effects for Pd, and COSMO to simulate the solvent effect in water. Human cancer cell lines A549 (lung carcinoma) and A431 (skin carcinoma) 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 were purchased from Bio-Connect BV. The FractionPREPTM Cell Fractionation kit was obtained from BioVision Incorporated.

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

The synthesis of the ligands MeL$^1$-MeL$^2$ and of their palladium complexes are shown in Scheme S1. The precursors HL$^1$-HL$^3$ were synthesized according to literature.$^3$
Scheme S1: Synthesis of ligands MeL\(^1\)-MeL\(^3\) and of their palladium complexes. Reaction condition: (a) CH\(_3\)I, KOt-Bu, DMF, room temperature, 24 h; (b) palladium(II) acetate, CH\(_3\)COOH, N\(_2\), 135 °C, 24 h; (c) Palladium(II) acetate, MeOH, 65 °C, 24 h.

Synthesis of ligand MeL\(^1\), MeL\(^2\), and MeL\(^3\)

The three ligands were synthesized in the same reaction conditions. The synthesis of MeL\(^1\) is described in detail below as an example.

MeL\(^1\). A mixture of its precursor HL\(^1\) (324.0 mg, 1.0 mmol), KOtBu (448.4 mg, 2.0 mmol), and CH\(_3\)I (282.0 mg, 0.1 mL, 2.0 mmol), was dissolved in DMF (10 mL) and stirred for 24 h at room temperature under an N\(_2\) atmosphere. Then the reaction solution was extracted with ethyl acetate (EtOAc) and water. NH\(_4\)Cl was added to the extracting solution for a good separation of the two layers. The crude product collected from the EtOAc layer was purified by silica gel chromatography using Pentane:EtOAc 4:1 (R\(_f\) = 0.25) to 2:1 (R\(_f\) = 0.35) as eluents. MeL\(^1\) was obtained in 47% yield (159.0 mg, 0.47 mmol). ESI-MS (cation): m/z calcd 339.2 (C\(_{22}\)H\(_{18}\)N\(_4\) + H\(^+\)), obsd 339.2; \(^1\)H NMR (400 MHz, Methanol-\(d_4\)) \(\delta\) 8.58 (2 H, dddd, J = 9.6, 4.9, 1.8, 0.9 Hz), 8.39 (1 H, dq, J = 8.0, 0.9 Hz), 7.92 (1 H, t, J = 2.0 Hz), 7.90 – 7.82 (3 H, m), 7.80 (1 H, ddd, J = 7.7, 1.8, 1.0 Hz), 7.63 (1 H, dd, J = 7.4, 0.8 Hz), 7.59 – 7.47 (2 H, m), 7.43 – 7.27 (3 H, m), 6.67 (1 H, dt, J = 8.5, 0.7 Hz), 3.61 (3 H, d, J = 0.6). \(^{13}\)CAPT-NMR (101 MHz, Methanol-\(d_4\)) \(\delta\) 159.6, 158.3, 158.1, 154.8, 150.4,
MeL²: This ligand were collected from the EtOAc layer was purified by silica gel chromatography using Pentane:EtOAc 4:1 (Rf = 0.25) to 2:1 (Rf = 0.35) as eluents. Yield 143.4 mg, 0.42 mmol, 42%; ESI-MS (cation): m/z calcd 339.2 (C₂₂H₁₈N₄ + H⁺), obsd 339.2; ¹H NMR (300 MHz, Methanol-d₄) 8.62 (1 H, d, J = 4.9 Hz), 8.38 (1 H, d, J = 8.0 Hz), 8.12 – 7.99 (2 H, m), 7.99 – 7.85 (2 H, m), 7.77 (2 H, td, J = 8.1, 6.8 Hz), 7.53 – 7.33 (6 H, m), 7.27 (1 H, d, J = 8.2 Hz), 3.79 (3 H, d, J = 1.7 Hz); ¹³C APT-NMR (75 MHz, Methanol-d₄) δ 149.9, 139.6, 139.3, 138.7, 128.0, 129.7, 127.8, 125.1, 124.0, 114.9, 114.9, 114.4, 35.0.

MeL³: This ligand was purified by alumina column chromatography with eluent Pentane:EtOAc (20:3, Rf = 0.3). Yield 56 mg, 0.17 mmol, 97%; ESI-MS (cation): m/z calcd 340.2 (C₂₂H₁₈N₄ + H⁺), obsd 340.3; ¹H NMR (400 MHz, Methanol-d₄) 8.64 – 8.59 (2 H, m), 8.36 (2 H, dt, J = 8.0, 1.1 Hz), 7.94 – 7.88 (4 H, m), 7.80 (2 H, dd, J = 8.3, 7.5 Hz), 7.44 – 7.34 (4 H, m), 3.80 (3 H, s). ¹³C APT-NMR (101 MHz, Methanol-d₄) δ 157.2, 156.1, 153.9, 148.6, 138.1, 137.3, 123.7, 121.2, 114.5, 113.8, 113.3, 35.0.

Synthesis of [PdMeL¹]OAc ([1]OAc)

A mixture of ligand MeL¹ (67.6 mg, 0.20 mmol) and and Pd(OAc)₂ (44.4 mg, 0.20 mmol) was dissolved in CH₃COOH (50 mL) and heating at 135 °C on N₂ atmosphere for 24 h. The solvent was rotary evaporated to obtain a yellow solid, which was washed with EtOAc (50 mL) and ether (30 mL), and dried under vacuum, to finally obtain [1]OAc as analytically pure product (Yield: 86 mg, 0.17 mmol, 86%). HRMS (cation): m/z calcd 443.0488 [C₂₂H₁₇N₄Pd]^+, obsd 443.0493. ¹H NMR (300 MHz, Methanol-d₄) 8.55 (1 H, d, J = 5.4 Hz), 8.35 (2 H, t, J = 8.3 Hz), 8.18 (1 H, td, J = 8.0, 1.5 Hz), 8.05 – 7.93 (2 H, m), 7.88 (2 H, td, J = 7.4, 1.3 Hz), 7.69 (1 H, ddd, J = 7.3, 5.6, 1.2 Hz), 7.45 – 7.30 (3 H, m), 7.25 (1 H, t, J = 7.8 Hz), 7.07 (1 H, dd, J = 8.4, 1.0 Hz), 3.59 (3 H, s), 1.90 (3 H, s). ¹³C APT-NMR (75 MHz, Methanol-d₄) δ 150.3, 149.4, 141.6, 141.4, 140.4, 124.8, 124.8, 121.5, 120.6, 119.7, 118.3, 116.3, 42.1. Elemental analysis calcd for [1]OAc + 6H₂O: C, 47.18; H, 5.28; N, 9.17; Found for [1]OAc + 6H₂O: 47.20, 5.22, 9.35.

Synthesis of complex [PdMeL²]OAc ([2]OAc)

[2]OAc was synthesized using the same method as for [2]OAc, but starting from ligand MeL² (67.6 mg, 0.2 mmol). The yield in [2]OAc was 88% (88.7 mg, 0.18 mmol). HRMS (cation): m/z calcd 443.0488 [C₂₂H₁₇N₄Pd]^+, obsd 443.0490. ¹H NMR (400 MHz, Methanol-d₄) 8.73 (1 H, dd, J = 5.9, 1.4 Hz), 8.39 – 8.32 (1 H, m), 8.22 (1 H, td, J = 7.8, 1.5 Hz), 8.18 – 8.10 (1 H, m), 8.01 (1 H, d, J = 7.7 Hz), 7.95 (1 H, t, J = 8.1 Hz), 7.67 (1 H, ddd, J = 7.3, 5.6, 1.4 Hz), 7.55 (2 H, dd, J = 11.6, 8.1 Hz), 7.52 – 7.48 (1 H, m), 7.20 (1 H, d, J = 8.5 Hz), 7.17 – 7.09 (3 H, m), 3.61 (3 H, s), 1.90 (3 H, s). ¹³C APT-NMR (101 MHz, Methanol-
δ 165.0, 158.0, 156.5, 152.7, 152.0, 151.8, 151.1, 142.2, 141.8, 141.7, 132.1, 130.5, 128.2, 127.0, 125.8, 125.3, 118.6, 118.1, 115.0, 114.7, 43.6. Elemental analysis calcd for [2]OAc + 2H2O: C, 53.49; H, 4.49; N, 10.40; Found for [2]OAc + 2H2O: 53.46, 4.53, 10.38.

Synthesis of complex [PdMeL3](OAc)2 ([3](OAc)2)

The mixture of ligand MeL3 (23.3 mg, 0.065 mmol) and Pd(OAc)2 (25.6 mg, 0.065 mmol) was dissolved in MeOH and heated at 65 °C under an N2 atmosphere for 24 h. The solvent was rotary evaporated to obtain a yellow solid, which was washed with EtOAc (50 mL) and then ether (30 mL), dried in vacuum to obtain [3](OAc)2 as analytically pure product (Yield: 29.3 mg, 0.054 mmol, 80%). HRMS (cation): m/z calcd 222.5254 [C21H17N3Pd]2+, obsd 222.5255. 1H NMR (400 MHz, Methanol-d4) 9.03 (2 H, d, J = 5.8 Hz), 8.75 (2 H, d, J = 8.1 Hz), 8.52 (6 H, dt, J = 16.2, 7.5 Hz), 8.04 (4 H, dd, J = 8.3, 5.2 Hz), 4.08 (3 H, s), 1.87 (6 H, s). 13C APT-NMR (101 MHz, Methanol-d4) δ 158.3, 155.4, 152.2, 151.2, 144.1, 143.8, 129.4, 125.9, 120.4, 119.9, 44.1. Elemental analysis calcd for [3](OAc)2 + 8H2O: C, 42.41; H, 5.55; N, 9.89; Found for [3](OAc)2 + 8H2O: 42.63, 4.92, 9.98.
Figure. S1 $^1$H NMR of MeL$^1$ in Methanol-$d_4$.

Figure. S2 $^{13}$C-APT NMR of MeL$^1$ in Methanol-$d_4$. 
Figure. S3 $^1$H NMR of MeL$^2$ in Methanol-$d_4$.

Figure. S4 $^{13}$C-APT NMR of MeL$^2$ in methanol-$d_4$. 

S7/S41
**Figure. S5** $^1$H NMR of MeL$^3$ in Methanol-$d_4$.

**Figure. S6** $^{13}$C-APT NMR of MeL$^3$ in methanol-$d_4$. 

S8/S41
Figure S7. $^1$H NMR of [PdMeL]$^1$OAc in Methanol-$d_4$.

Figure S8. $^{13}$C-APT NMR of [PdMeL]$^1$OAc in Methanol-$d_4$. 
Figure S9. $^1$H NMR of [PdMe$_2$L$_2$]OAc in Methanol-$d_4$.

Figure S10. $^{13}$C-APT NMR of [PdMe$_2$L$_2$]OAc in Methanol-$d_4$. 
Figure S11. $^{1}$H NMR of $[\text{PdMeL}^3](\text{OAc})_2$ in Methanol-$d_4$.

Figure S12. $^{13}$C-APT NMR of $[\text{PdMeL}^3](\text{OAc})_2$ in Methanol-$d_4$.
Figure S13. The aromatic region of the $^1$H NMR spectrum of complexes $[1]^+$-[3]$^{2+}$ at low (2 mg/mL) and high (7 mg/mL) concentration. Solvent: CD$_3$OD.
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 Kα radiation (\(\lambda = 0.71073\) Å) for [1]PF₆ and [3](BF₄)₂, and with Cu Kα radiation (\(\lambda = 1.54178\) Å) for [2]PF₆ 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 performed 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 (unless otherwise specified) using the instructions AFIX 43 or AFIX 137 with isotropic displacement parameters having values 1.2 or 1.5 \(U_{eq}\) of the attached C atoms. The structures of the three complexes have been deposited to the CCDC with the deposition number 1980994-1980996.

[1]PF₆: The structure is mostly ordered. There is some substitutional disorder at the positions occupied by the atoms N2/C17’ and C17/N2’ as both positions are occupied by a mixture of N or C atom (the occupancy for each site must be 1). The occupancy factor of the major component of the disorder for N2 and C17 refines to 0.58(2).

[2]PF₆: The structure is mostly ordered. There is some substitutional disorder at the positions occupied by the atoms N1/C22’ and C22/N1’ as both positions are occupied by a mixture of N or C atom (the occupancy for each site must be 1). The occupancy factor of the major component of the disorder for N1 and C22 refines to 0.82(3). The crystal that was mounted on the diffractometer was non-merohedrally twinned. The two components are related by a twofold rotational axis along the reciprocal 0.9591a* – 0.1285b* – 0.2523c* direction. The structure refinement was processed using the HKLF 5 instruction. The BASF scale factor refines to 0.0755(12).

[3](BF₄)₂: The structure is ordered

Table S1. Crystallographic Data for [1]PF₆, [2]PF₆, [3](BF₄)₂.

| [1]PF₆       |
|--------------|
| Crystal data |
| Chemical formula | C₂₂H₁₇N₄Pd·F₆P |
| \(M_r\)       | 588.77         |
| Crystal system, | Triclinic, P-1 |

S13/S41
space group

Temperature (K) 110

\(a, b, c (\text{Å})\) 7.13321 (15), 12.3984 (3), 12.5417 (3)

\(\alpha, \beta, \gamma (\text{o})\) 111.159 (2), 95.1949 (18), 100.328 (2)

\(V (\text{Å}^3)\) 1002.98 (4)

\(Z\) 2

Radiation type Mo \(K\alpha\)

\(\mu (\text{mm}^{-1})\) 1.08

Crystal size (mm) 0.25 \(\times\) 0.06 \(\times\) 0.03

Data collection

Diffractometer SuperNova, Dual, Cu at zero, Atlas

Absorption Gaussian

correction 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.

\(T_{\text{min}}, T_{\text{max}}\) 0.745, 1.000

No. of measured, independent and observed \([I > 2\sigma(I)]\) reflections 15557, 4607, 4258

\(R_{\text{int}}\) 0.027

\((\sin \theta/\lambda)_{\text{max}} (\text{Å}^{-1})\) 0.650

Refinement

\(R[F^2 > 2\sigma(F^2)]\), \(wR(F^2), S\) 0.025, 0.055, 1.06
No. of reflections 4607
No. of parameters 309
H-atom treatment H-atom parameters constrained
Δρ_{max}, Δρ_{min} (e Å^{-3}) 0.51, -0.48

| Crystal data |
|--------------|
| Chemical formula | C_{22}H_{17}N_{4}Pd·F_{6}P |
| M_t | 588.77 |
| Crystal system, space group | Triclinic, P-1 |
| Temperature (K) | 110 |
| a, b, c (Å) | 7.0794 (2), 12.4486 (4), 12.4689 (4) |
| α, β, γ (°) | 111.199 (3), 98.922 (3), 94.144 (2) |
| V (Å³) | 1002.24 (6) |
| Z | 2 |
| Radiation type | Cu Kα |
| μ (mm⁻¹) | 8.93 |
| Crystal size (mm) | 0.15 × 0.05 × 0.03 |

| Data collection |
|----------------|
| Diffractometer | SuperNova, Dual, Cu at zero, Atlas |
| Absorption correction | CrysAlis PRO 1.171.39.29c (Rigaku Oxford Diffraction, 2017) |
| Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid. (Clark, R. C. & Reid, J. S. (1995). Acta Cryst. A51, 887-897) |
| Empirical absorption correction using spherical harmonics,
implemented in SCALE3 ABSPACK scaling algorithm.

$T_{\text{min}}, T_{\text{max}}$ 0.384, 0.824

No. of measured, independent and observed $[I > 2\sigma(I)]$ reflections

$R_{\text{int}}$ 0.017

$(\sin \theta/\lambda)_{\text{max}}$ (Å$^{-1}$) 0.616

Refinement

$R[F^2 > 2\sigma(F^2)]$, 0.020, 0.049, 0.97

$wR(F^2), S$

No. of reflections 4342

No. of parameters 310

No. of restraints 0

H-atom treatment H atoms treated by a mixture of independent and constrained refinement

$\Delta \rho_{\text{max}}, \Delta \rho_{\text{min}}$ (e Å$^{-3}$) 0.35, -0.63

[3](BF$_4$)$_2$

Crystal data

Chemical formula C$_{21}$H$_{17}$N$_5$Pd·2(BF$_4$)

$M_f$ 619.42

Crystal system, space group Triclinic, $P$-1

Temperature (K) 110

$a, b, c$ (Å) 8.7303 (3), 9.6154 (3), 13.7013 (5)

$\alpha, \beta, \gamma$ (°) 77.129 (3), 76.604 (3), 83.396 (3)
| Property                      | Value                  |
|------------------------------|------------------------|
| $V (\text{Å}^3)$             | 1088.31 (7)            |
| $Z$                          | 2                      |
| Radiation type               | Mo $K\alpha$           |
| $\mu$ (mm$^{-1}$)            | 0.94                   |
| Crystal size (mm)            | 0.13 × 0.06 × 0.03     |

**Data collection**

- **Diffractometer**: SuperNova, Dual, Cu at zero, Atlas
- **Absorption correction**: Gaussian
- **CrystAlis 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.

- $T_{\text{min}}, T_{\text{max}}$: 0.859, 1.000
- No. of measured, independent and observed [$I > 2\sigma(I)$] reflections: 19782, 4998, 4289
- $R_{\text{int}}$: 0.048
- $(\sin \theta/\lambda)_{\text{max}}$ (Å$^{-1}$): 0.650

**Refinement**

- $R[F^2 > 2\sigma(F^2)]$, $wR(F^2), S$: 0.034, 0.071, 1.06
- No. of reflections: 4998
- No. of parameters: 335
- H-atom treatment: H-atom parameters constrained
$\Delta \rho_{\text{max}}, \Delta \rho_{\text{min}}$ (e Å$^{-3}$) 0.85, -0.52

Computer programs: CrysAlis PRO 1.171.39.29c (Rigaku OD, 2017), SHELXS2014/7 (Sheldrick, 2015), SHELXL2014/7 (Sheldrick, 2015), SHELXTL v6.10 (Sheldrick, 2008).
4. DFT calculation of HOMO-LUMO orbitals and TDDFT-calculated spectra of metal complexes

**Figure S14.** DFT calculation of HOMOs (bottom) and LUMOs (top) orbitals of [1]^+/[3]^{2+}; occupied orbitals (HOMO) have red and blue lobes, and unoccupied orbitals (LUMO) brown and cyan lobes. Element color code: grey = C; orange = Pd; blue = N; white = H. Level of theory: ADF/DFT/PBE0/TZP/COSMO(water).

**Table S2.** HOMO and LUMO energies, energy gap (ΔE) of complexes [1]^+-[3]^{2+}.

| Complex | HOMO/eV | LUMO/eV | ΔE/eV |
|---------|---------|---------|-------|
| [1]^+   | -6.24   | -2.41   | 3.83  |
| [2]^+   | -6.58   | -2.46   | 4.11  |
| [3]^{2+}| -6.95   | -2.82   | 4.13  |
**Figure S15.** TDDFT-calculated spectra for palladium complexes $[{1}^+]$-$[{3}^{2+}]$. Level of theory: ADF/TDDFT/PBE0/TZP/COSMO (water).

**Table S3.** Cartesian coordinates (Å) for DFT-optimized geometries of palladium complexes $[{1}^+]$-$[{2}^+]$.

|       | [1]$^+$ |   |   | [2]$^+$ |   |   |
|-------|---------|---|---|---------|---|---|
|       | x       | y | z | x       | y | z |
| Pd    | 1.129264| 3.612537| 3.368055 | Pd    | 1.129264| 3.612537| 3.368055 |
| N     | 3.083289| 4.061773| 3.702159 | C      | 3.083289| 4.061773| 3.702159 |
| C     | 1.096968| 5.537636| 2.758949 | N      | 1.096968| 5.537636| 2.758949 |
| C     | 1.660038| 0.888034| 4.78826  | C      | 1.660038| 0.888034| 4.78826  |
| H     | 2.611182| 1.331329| 5.042771 | H      | 2.611182| 1.331329| 5.042771 |
| C     | 1.346406| -0.38751 | 5.214949 | C      | 1.346406| -0.38751 | 5.214949 |
| H     | 2.064282| -0.95066 | 5.79695  | H      | 2.064282| -0.95066 | 5.79695  |
| C     | 0.106039| -0.90699 | 4.881869 | C      | 0.106039| -0.90699 | 4.881869 |
| H     | -0.18046| -1.90474 | 5.192633 | H      | -0.18046| -1.90474 | 5.192633 |
| C     | -0.77442| -0.12454 | 4.159    | C      | -0.77442| -0.12454 | 4.159    |
| H     | -1.75644| -0.50254 | 3.912618 | H      | -1.75644| -0.50254 | 3.912618 |
| C     | -0.39915| 1.155363| 3.763721 | C      | -0.39915| 1.155363| 3.763721 |
| C     | -1.31322| 2.06053 | 3.042232 | C      | -1.31322| 2.06053 | 3.042232 |
| C     | -2.56022| 1.67721 | 2.604427 | C      | -2.56022| 1.67721 | 2.604427 |
| H     | -2.92571| 0.671093| 2.750372 | H      | -2.92571| 0.671093| 2.750372 |
| C     | -3.35236| 2.63295 | 1.95359  | C      | -3.35236| 2.63295 | 1.95359  |
|        | x         | y         | z         |
|--------|-----------|-----------|-----------|
| Pd     | 1.129264  | 3.612537  | 3.368055  |
| N      | 3.083289  | 4.061773  | 3.702159  |

**Table S4.** Cartesian coordinates (Å) for DFT-optimized geometries of complex [3]$^{2+}$. 
|  |  |  |
|---|---|---|
| N | 1.096968 | 5.537636 | 2.758949 |
| C | 1.660038 | 0.888034 | 4.78826 |
| H | 2.611182 | 1.331329 | 5.042771 |
| C | 1.346406 | -0.38751 | 5.214949 |
| H | 2.064282 | -0.95066 | 5.79695 |
| C | 0.106039 | -0.90699 | 4.881869 |
| H | -0.18046 | -1.90474 | 5.192633 |
| C | -0.77442 | -0.12454 | 4.159 |
| H | -1.75644 | -0.50254 | 3.912618 |
| C | -0.39915 | 1.155363 | 3.763721 |
| C | -1.31322 | 2.06053 | 3.042232 |
| C | -2.56022 | 1.67721 | 2.604427 |
| H | -2.92571 | 0.671093 | 2.750372 |
| C | -3.35236 | 2.63295 | 1.95359 |
| H | -4.33907 | 2.360981 | 1.595862 |
| C | -2.88174 | 3.902 | 1.772563 |
| H | -3.47316 | 4.661381 | 1.277213 |
| C | -1.58211 | 4.266854 | 2.228867 |
| C | -0.02962 | 6.126999 | 2.274306 |
| C | 0.03078 | 7.519892 | 1.988035 |
| H | -0.86713 | 7.985985 | 1.60362 |
| C | 1.178879 | 8.230962 | 2.194247 |
| H | 1.208125 | 9.292876 | 1.975149 |
| C | 2.319934 | 7.58818 | 2.685412 |
| H | 3.236647 | 8.13752 | 2.849399 |
| C | 2.25082 | 6.240782 | 2.957999 |
| C | 3.362809 | 5.424572 | 3.452673 |
| C | 4.650207 | 5.935101 | 3.634463 |
| H | 4.857607 | 6.983468 | 3.449649 |
| C | 5.682005 | 5.104361 | 4.041492 |
| H | 6.679271 | 5.50622 | 4.182272 |
| C | 5.432544 | 3.75349 | 4.248244 |
| H | 6.239177 | 3.089347 | 4.541797 |
| C | 4.147504 | 3.245825 | 4.074666 |
|  |  |  |
|---|---|---|
| H | 4.008053 | 2.180907 | 4.22166 |
| N | -1.20835 | 5.535927 | 2.010939 |
| N | -0.84565 | 3.317513 | 2.851833 |
| N | 0.828409 | 1.64131 | 4.065571 |
| C | -2.40685 | 6.58238 | 1.334279 |
| H | -2.11809 | 7.600562 | 1.119054 |
| H | -3.38924 | 6.573958 | 1.782879 |
| H | -2.6564 | 6.247374 | 0.338339 |
5. Phosphorescence lifetime spectra of complexes [1]⁺-[3]²⁺

![Phosphorescence lifetime spectra of complexes](image)

**Figure S16.** The phosphorescence lifetime spectra and fit curve of complexes [1]⁺-[3]²⁺ in water under air atmosphere at room temperature. Fit equation: $y = y_0 + A_1 \cdot \exp\left(-\frac{(x-x_0)}{\tau_1}\right)$ ([1]⁺ and [2]⁺), $y = y_0 + A_1 \cdot \exp\left(-\frac{(x-x_0)}{\tau_1}\right) + A_2 \cdot \exp\left(-\frac{(x-x_0)}{\tau_2}\right)$ ([3]²⁺). Excitation wavelength: 375 nm. The data were analyzed via OriginPro 9.1.
6. Derived count rate and size distribution of metal complexes by Dynamic Light Scattering (DLS)

Table S5. The derived countrate (kcps) values of complexes in different solutions and different concentrations.

| complex | H$_2$O | PBS | Opti-MEM with FCS | Opti-MEM without FCS | Opti-MEM with BSA |
|---------|--------|-----|-------------------|-----------------------|-------------------|
| Solution only | 54 | 40 | 894 | 107 | 8257 |
| [1]$^*$ | 5 µM | 37 | 65 | 2268 | 1936 |
| 50 µM | 146 | 187 | 19144 | 18970 | 14105 |
| [2]$^*$ | 5 µM | 30 | 58 | 2775 | 5657 |
| 50 µM | 67 | 61 | 22311 | 25377 | 13359 |
| [3]$^*$ | 5 µM | 56 | 60 | 927 | 136 |
| 50 µM | 79 | 21 | 930 | 101 | 8056 |

Figure S17. Size distributions according to DLS of solution of [1]OAc-[3]OAc at 5 or 50 µM in different solvents.
Figure S18. DLS size distribution (left and middle) and derived count rate (right) of [1]OAc (50 µM) in Opti-MEM complete medium at different pH.
7. Stability of metal complexes in different bio-relevant solvents.
Figure S19. Time evolution of the absorbance spectra of solutions of [1][1]-[3][2] in H$_2$O (a), PBS (b), cell medium with FCS (c), cell medium without FCS (d), H$_2$O with GSH (200 µM, e) and H$_2$O with ascorbic acid (200 µM, f) at 310 K. Concentration 50 µM. The color change of the spectra indicates the time, with black corresponding to the first curve (0 h) and red to the last one (24 h).
8. TEM images of complexes from evaporated MilliQ water solutions.

**Figure S20.** TEM images of samples prepared from evaporated MilliQ water solutions of [1]OAc, [2]OAc, and [3](OAc)$_2$ (50 µM). Scale bar: 2 µm (top) and 200 nm (bottom).

**Figure S21.** The Cryo-TEM images of complexes [1]$^+$-[2]$^+$ (50 µM) in the Opti-MEM medium with or without FCS.
9. DFT model of supramolecular dimers of the palladium complexes \{[1]^+\}_2 and \{[2]^+\}_2

![Diagram of DFT-optimized dimers \{[1]^+\}_2 and \{[2]^+\}_2.]

**Figure S22.** Structures of the DFT-optimized dimers \{[1]^+\}_2 (left) and \{[2]^+\}_2 (right).

**Table S6.** Cartesian coordinates (Å) for DFT-optimized geometries of the dimers of palladium complex \{[1]^+\}_2 and \{[2]^+\}_2.

|          | \{[1]^+\}_2 |          | \{[2]^+\}_2 |
|----------|-------------|----------|-------------|
|          | x           | y        | z           | x           | y        | z           |
| H        | 4.207466    | 3.003156 | 0.799992    | C           | 3.056994    | 3.961661    | 9.424639    |
| C        | 3.299029    | 4.826001 | 1.520196    | H           | 2.865631    | 4.563963    | 10.298888   |
| H        | 3.116947    | 5.243986 | 0.537146    | C           | 2.717595    | 2.627671    | 9.421484    |
| C        | 2.946458    | 5.532135 | 2.652147    | H           | 2.275251    | 2.185040    | 10.306339   |
| H        | 2.500117    | 6.513558 | 2.568790    | C           | 2.925405    | 1.866943    | 8.288880    |
| C        | 3.172837    | 4.981355 | 3.906680    | H           | 2.661809    | 0.819797    | 8.269697    |
| C        | 2.935123    | 5.652891 | 5.174799    | C           | 3.420746    | 2.482448    | 7.155306    |
| C        | 2.340005    | 6.901043 | 5.282781    | Pd          | 4.252664    | 4.552187    | 5.365312    |
| H        | 1.960532    | 7.422416 | 4.413101    | C           | 5.003516    | 5.094926    | 2.433692    |
| C        | 2.206111    | 7.466728 | 6.536857    | H           | 5.150154    | 4.028093    | 2.389253    |
| H        | 1.704667    | 8.419339 | 6.655701    | C           | 5.176044    | 5.879304    | 1.312323    |
| C        | 2.689809    | 6.814695 | 7.655824    | H           | 5.437866    | 5.419274    | 0.368636    |
| H        | 2.530625    | 7.274140 | 8.621089    | C           | 5.000905    | 7.245208    | 1.428897    |
| C        | 3.321255    | 5.567018 | 7.550745    | H           | 5.109925    | 7.897160    | 0.570462    |
| C        | 4.100510    | 5.892361 | 9.835128    | C           | 4.695310    | 7.770504    | 2.669108    |
| H        | 3.235084    | 5.994619 | 10.494504   | H           | 4.577904    | 8.837708    | 2.787601    |

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|   |     |       |       |   |     |       |       |
|---|-----|-------|-------|---|-----|-------|-------|
| N | 7.158909 | 4.562495 | 7.489786 | N | 6.660073 | 2.168413 | 7.779103 |
| C | 7.354436 | 2.507776 | 5.901081 | N | 7.103623 | 1.268674 | 5.328528 |
| N | 7.199283 | 1.650911 | 3.603659 | N | 7.297456 | 1.896265 | 3.059561 |
| N | 6.294593 | 6.253219 | 4.724992 | N | 7.597596 | 3.951487 | 4.246974 |
10. Photophysical properties of [1]OAc and [Ru(bpy)$_3$]Cl$_2$ in Opti-MEM complete medium

Direct detection of $^1$O$_2$ by emission spectroscopy at 1270 nm is only possible in our setup in CD$_3$OD and impossible in non-deuterated aqueous solutions, where the lifetime of $^1$O$_2$ is too short. We hence used a singlet oxygen probe, 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABMDMA) to determine the generation of singlet oxygen in Opti-MEM complete medium, i.e. in aggregated conditions, before and after light (450 nm) irradiation. In Opti-MEM complete medium, ABMDMA absorbs light at 378 nm, but it forms an endoperoxide in the presence of $^1$O$_2$, leading to a loss of conjugation and thus a decrease in its absorbance at 378 nm. As shown in Figure S27, ABMDMA (100 µM) itself was stable in the dark, and only slowly decreased under light irradiation (450 nm) for 1 min. When mixed with [1]OAc or [Ru(bpy)$_3$]Cl$_2$ (50 µM), in the dark it was still stable; but upon irradiation in the same conditions, the absorbance of ABMDMA decreased, much faster (by a factor ~3.5) for [1]OAc than for [Ru(bpy)$_3$]Cl$_2$.

To quantify this observation, we assumed that the absorbance at 450 nm of the solution of [1]OAc in medium was essentially due to light absorption by [1]$^+$ (and thus excited state generation); in other words, we neglected scattering by the nanoaggregates at this (rather high) wavelength. Then, the reference value for the $^1$O$_2$ generation quantum yield $\phi_\Delta$ of [Ru(bpy)$_3$]Cl$_2$ (0.25) is only known experimentally in D$_2$O.$^4$ The $^1$O$_2$ QY in pure H$_2$O (0.16) is usually calculated from that value, using the different values, in D$_2$O and H$_2$O, of the lifetime of [Ru(bpy)$_3$]Cl$_2$ in absence of dioxygen, $\tau_0$ (987 vs. 635 ns, respectively), and the second-order quenching rate constants by O$_2$, $k_\text{q}$ ($3.38 \times 10^9$ vs. $2.55 \times 10^9$ M$^{-1}$s$^{-1}$, respectively). Then again, medium is not pure water, and in particular the high salt concentration in medium decreases significantly the O$_2$ partial pressure at equilibrium from 21% in pure water at the equilibrium to 18% (pO$_2$ 140 mmHg) in medium at the equilibrium.$^5$ From these values, and using the equation for calculating $P_T^{O_2}$ the fraction of $^3$MLCT states quenched by dioxygen:

$$P_T^{O_2} = \frac{\tau_0 \cdot k_\text{q} \cdot [O_2]}{1 + \tau_0 \cdot k_\text{q} \cdot [O_2]}$$

Assuming that $\tau_0$ and $k_\text{q}$ do not vary between pure water and medium, then $P_T^{O_2} = 0.278$ and hence $\phi_\Delta$=0.14 in medium for [Ru(bpy)$_3$]Cl$_2$. Using this as reference value, and the different slopes of the evolution of the absorbance at 378 nm vs. irradiation time for [1]OAc and [Ru(bpy)$_3$]Cl$_2$, we obtained a $^1$O$_2$ generation quantum yield $\phi_\Delta$ of 0.73 for the aggregates of [1]OAc in medium, which is more or less equal, consider our assumptions, to the value for the monomer measured in CD$_3$OD by spectroscopic detection at 1270 nm (0.78).

Figure S23. The absorbance (a) and emission spectra (b) of [1]OAc (50 µM) in water and Opti-MEM complete medium.
Figure S2. The absorbance of ABMDMA Opti-MEM complete solution (100 µM) in the absence or presence of [Ru(bpy)₃]Cl₂ (50 µM) under dark or blue light (450 nm) irradiation. (b) Absorbance time evolution and linear fit curve at 378 nm of ABMDMA Opti-MEM complete solution (100 µM) in the absence or presence of [1]OAc (50 µM), [Ru(bpy)₃]Cl₂ (50 µM) under blue light irradiation. The baseline for these spectra was a solution of [1]OAc (50 µM) or [Ru(bpy)₃]Cl₂ (50 µM) in Opti-MEM medium without ABMDMA.
11. Cytotoxicity dose-response curves of metal complexes in normoxic and hypoxic conditions

Figure S25. Dose-response curves for A549 and A431 cancer cells incubated with complexes [1]OAc-[3](OAc)_2 and cisplatin, either in the dark (black data points) or upon blue light irradiation (5 minutes, 5.66 mW cm\(^{-2}\), 1.7 J cm\(^{-2}\), blue data points), under normoxic condition (37 °C atmosphere, 21% O\(_2\) and 7.0% CO\(_2\)).

Figure S26. Dose-response curves for A549 and A431 cancer cells incubated with complexes [1]OAc-[3](OAc)_2, cisplatin, 5-ALA and Rose bengal either in the dark (black data points) or upon blue light irradiation (455 nm, 8 min, 3.54 mW cm\(^{-2}\), 1.7 J cm\(^{-2}\), blue data points) under hypoxic condition (37 °C atmosphere, 1% O\(_2\) and 7.0% CO\(_2\)).
Figure S27. Graphical representation of the EC50 values of complexes [1]OAc-[3](OAc)2 in A549 and A431 in the dark or upon blue light irradiation, in normoxic vs. hypoxic conditions. (irradiation condition: normoxic 455 nm, 5 minutes, 5.66 mW cm⁻², 1.7 J cm⁻², hypoxic 455 nm, 8 min, 3.54 mW cm⁻², 1.7 J cm⁻²).
11. Uptake and subcellular fractionation studies with A549 cells

Table S7. Palladium cellular uptake according to ICP-MS analysis in the different fractions of A549 cells treated with $[\text{1}]\text{OAc}-[\text{3}]\text{(OAc)}_2$ (1 µM) in the dark after 24 h.

| Complex | Treatment (ng Pd) | Metal uptake (ng Pd/million cells) | Metal uptake efficiency (%) | Fractions | Metal distribution (ng Pd/million cells) | Relative metal distribution (%) |
|---------|-------------------|-----------------------------------|----------------------------|-----------|-----------------------------------------|--------------------------------|
| $[\text{1}]\text{OAc}$ | 212.84 | 19±7 | 5.2 | cytosol | 0.7±0.1 | 3.5 |
| | | | | membranes | 0.23±0.03 | 1.1 |
| | | | | nucleus | 0.23±0.03 | 1.1 |
| | | | | cytoskeleton | 17±3 | 94.3 |
| $[\text{2}]\text{OAc}$ | 212.84 | 14±4 | 9.8 | cytosol | 0.45±0.05 | 3.3 |
| | | | | membranes | 0.68±0.09 | 5.1 |
| | | | | nucleus | 0.34±0.04 | 2.5 |
| | | | | cytoskeleton | 12±2 | 89.1 |
| $[\text{3}]\text{(OAc)}_2$ | 212.84 | 1.7±0.2 | 0.8 | cytosol | 0.42±0.09 | 23.3 |
| | | | | membranes | 0.28±0.06 | 15.6 |
| | | | | nucleus | 0 | 0 |
| | | | | cytoskeleton | 1.1±0.2 | 61.1 |
12. Cell death mode according to FACS

Figure S28. Annexin V/propidium iodide double staining FACS data for A549 cells after treatment with cisplatin (15 µM) and complexes [1]OAc-[3](OAc)₂ (15 µM) in the dark or upon blue light irradiation (455 nm, 5 minutes, 5.66 mW cm⁻², 1.7 J cm⁻²).
13. *In vivo* body weight after treatment

**Scheme S2.** The sketch of *in vivo* experiments for complexes [1]+ and [2]+.

**Figure S29.** Bodyweight of mice treated with [1]OAc and [2]OAc and control groups.
14. Reference

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