Electronic Supplementary Information

High-Affinity Sequence-Selective DNA binding by Iridium(III) polypyridyl organometallopeptides

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General

All reagents were acquired from commercial sources: Dimethylformamide (DMF) and Trifluoroacetic acid (TFA) were purchased from Scharlau, CH_2Cl_2 from Panreac and CH_3CN from Merck. All peptide synthesis reagents, namely the coupling agents HBTU (O-Benzotriazole-*N,N,N′,N′*-tetramethyl-uronium-hexafluorophosphate) and HATU (2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate methanaminium), and Fmoc amino acid derivatives were purchased from *GL Biochem* (Shanghai) Ltd. All other chemicals were purchased from Sigma-Aldrich or Fluka. All solvents were dry and of synthesis grade, unless specifically noted. $IrCl_3$ and 2-phenylpyridine (ppy) were purchase from *Sigma-Aldrich*. Reactions were followed by analytical RP-HPLC with an Agilent 1100 series LC/MS using a *Luna C18* (250 x 4.60 mm) analytical column from Phenomenex. Standard conditions for analytical RP-HPLC consisted on a linear gradient from 30% to 95% of solvent B for 30 min at a flow rate of 1 mL/min (A: water with 0.1% TFA, B: acetonitrile with 0.1% TFA). Compounds were detected by UV absorption at 222, 254 and 310 nm. High-Performance Liquid Chromatography (HPLC) was performed using an *Agilent 1100* series Liquid Chromatograph Mass Spectrometer system. Analytical HPLC was run using a *Luna C18* (250 x 4.60 mm) reverse phase analytical column; compounds were detected by UV absorption at 222, 254 and 310 nm. The purification of the peptides was performed on a *Luna C18* (250 x 10 mm) semi-preparative reverse phase column from Phenomenex. The standard gradient used for analytical and semi-preparative HPLC was 70:30 to 5:95 over 30 min (water/acetonitrile, 0.1% TFA). Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an *Agilent 1100* Series LC/MSD VL model in positive scan mode using direct injection of the purified peptide solution into the MS. Peptides purification was performed by semi-preparative RP-HPLC with an Agilent 1100 series Liquid Chromatograph using a Luna 5u C_{18} 100A (5 µm, 10 × 250 mm) reverse-phase column from Phenomenex. Standard conditions for analytical and semi-preparative RP-HPLC consisted on an isocratic regime during the first 5 min, followed by a linear gradient from 30% to 95% of solvent B for 30 min (A: water 0.1% TFA, B: acetonitrile 0.1% TFA). Compounds were det'ected by UV absorption (222 nm) and by ESI^+−MS. The fractions containing the products were freeze-dried, and their identity was confirmed by ESI^+−MS and MALDI-TOF. Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MS model in positive scan mode using direct injection of the purified peptide solution into the MS. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI/MS) was performed with a Bruker Autoflex MALDI/TOF model in positive scan mode by direct irradiation of the matrix-absorbed peptide. Luminescence experiments were made with a *Jobin-Yvon Fluoromax-3* fluorescence spectrometers (DataMax 2.20), coupled to a *Wavelength Electronics* LFI−3751 temperature controller. All measurements were made with a *Hellma* semi-micro cuvette (114F-QS) at 20 ºC.

Synthesis of the unnatural coordinating residue Fmoc-βAla-bpy-OH (1)

The coordinating residue Fmoc-βAla-bpy-OH (**1**) was synthetized following a procedure previously reported by our research group.[1]

Synthesis of the peptide ligands and Ir(III) organometallopeptides

Synthesis of the peptide ligands

C-terminal amide peptides were synthesized by following standard SPPS protocols on a 0.1 mmol scale using a 0.45 mmol g^{-1} Fmoc-Rink-amide resin. Arginines were coupled, in 10-fold excess (*vs*. mmol of resin load), by using O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) as an activating agent.

Fmoc-βAla-bpy-OH (1) was coupled in 5-fold excess by using 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-b]pyridinium3-oxide hexafluorophosphate (HATU) as an activating agent.

Couplings were conducted for 1 h. Deprotection of the temporal Fmoc protecting-group was performed with 20% piperidine in DMF for 15 min. Test cleavages were performed at a 5 mg scale for 2 h with CH_2Cl_2 (50 mL), H_2O (25 mL), triisopropylsilane (TIS; 25 mL), and TFA (900 mL) (~1 mL of cocktail for 100 mg of resin).

Synthesis of the Ir(III) organometallopeptides

Once the peptide ligands were synthesized, the resin was suspended in DMF (6 mL) in the dark for 15 min. $[Ir(ppy)_2Cl]_2$ was added in the selected ratio (1.0, 2.0 or 3.0 equivalents for **Ir**/**Ir-R$_8$**, **Ir$_2$**/**Ir$_2$-R$_8$** and **Ir$_3$**/**Ir$_3$-R$_8$**/**Ir-R$_3$-Ir-R$_3$-Ir-R$_3$** respectively), and the resulting mixture was stirred under argon for 8 hours at 80 ºC. The resin was filtered, washed with DMF and CH_2Cl_2 and dried. The metallopeptide was cleaved with the TFA cocktail; the resin was filtered and the filtrate was successively concentrated, precipitated, dried, redissolved in acetonitrile/water 1:5 and purified by semi-preparative HPLC to give the desired product. $[Ir(ppy)_2Cl]_2$ was synthetized from $IrCl_3$ following a reported procedure.[2]

General procedure for cleavage-deprotection

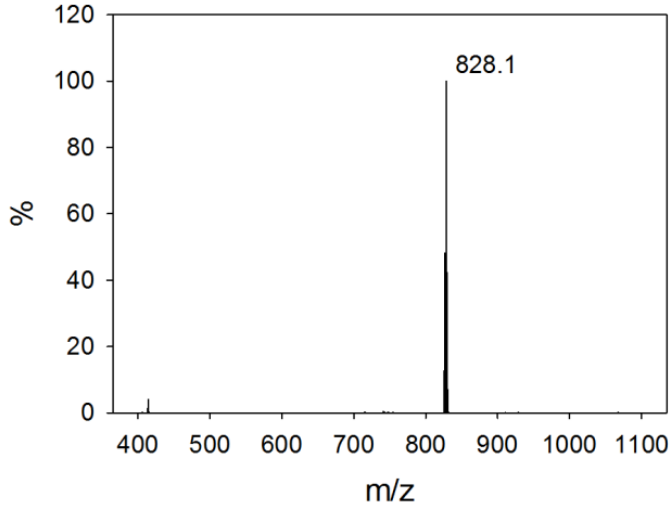
A resin-bound peptide dried overnight (0.025 mmol) was placed in a 50 mL falcon tube, to which 3 mL of the cleavage cocktail (50 µL CH_2Cl_2, 25 µL of H_2O, 25 µL of TIS (Triisopropylsilane) and 940 TFA µL) were added. The resulting suspension was shaken for 3 h. The resin was filtered, and the TFA filtrate was concentrated with a nitrogen current to an approximate volume of 1 mL, which was added to ice-cold diethyl ether (10 mL). After 10 min, the precipitate was centrifuged and washed again with 15 mL of ice-cold ether. The solid residue was dried under argon and redissolved in acetonitrile/water 1:5 (1 mL) and purified by

semi-preparative reverse-phase HPLC. The collected fractions were lyophilized and stored at −20 °C. Their identity was confirmed by ESI^+-MS and MALDI-TOF.

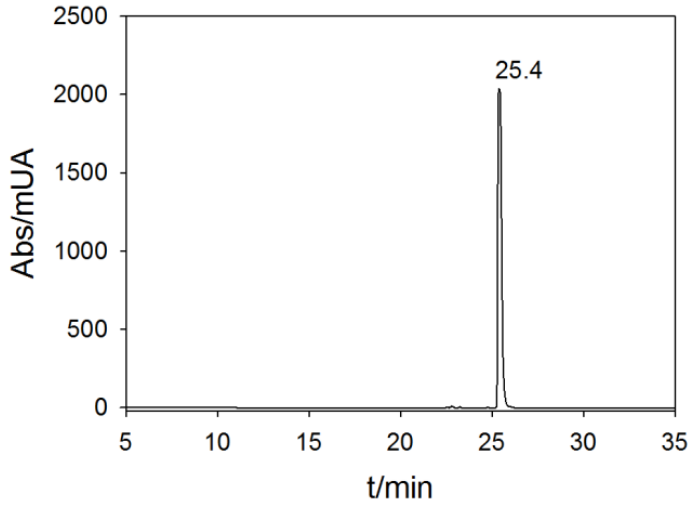
Mass spectra and HPLC chromatograms of the Ir(III) organometallopeptides

Ir

MS(ESI) (m/z): calculated ($[M]^+$, $[C_{38}H_{33}IrN_7O_3]^+$): 828.23, found: 828.1.

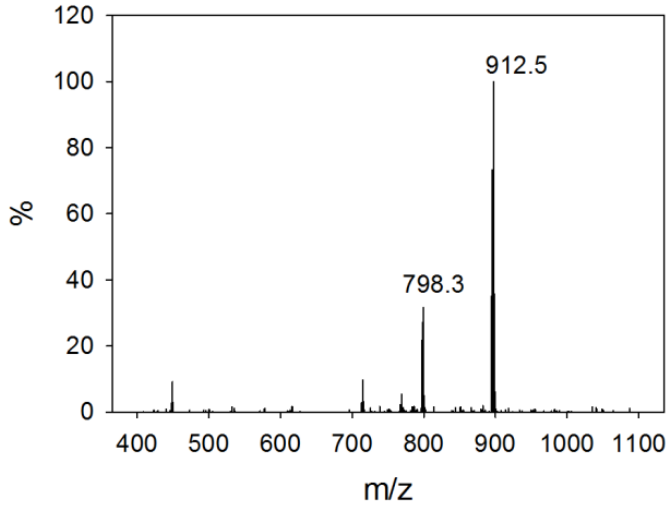


HPLC chromatogram: 1-75 %B, t_R=25.4´.

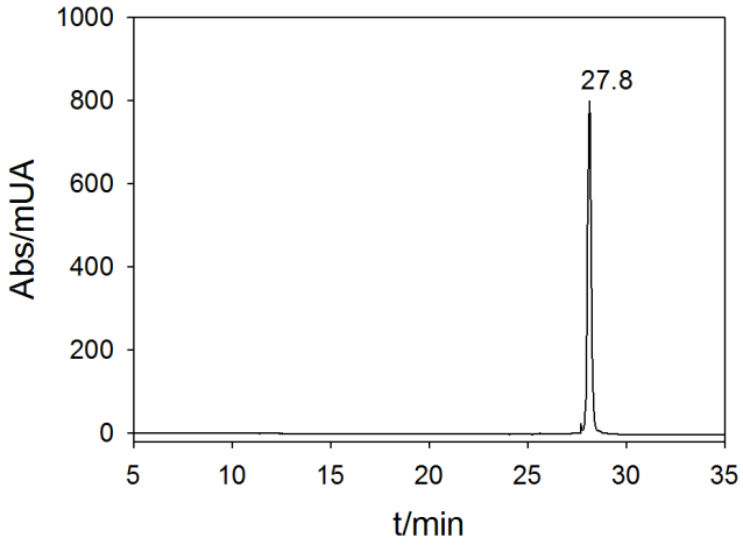


Ir₂

MS(ESI) (m/z): calculated ($[M+2TFAH]^{2+}$, $[C_{78}F_6H_{63}Ir_2N_{13}O_9]^{2+}$): 912.7, found: 912.5; calculated ($[M]^{2+}$, $[C_{74}H_{61}Ir_2N_{13}O_5]^{2+}$): 798.7, found: 798.3.

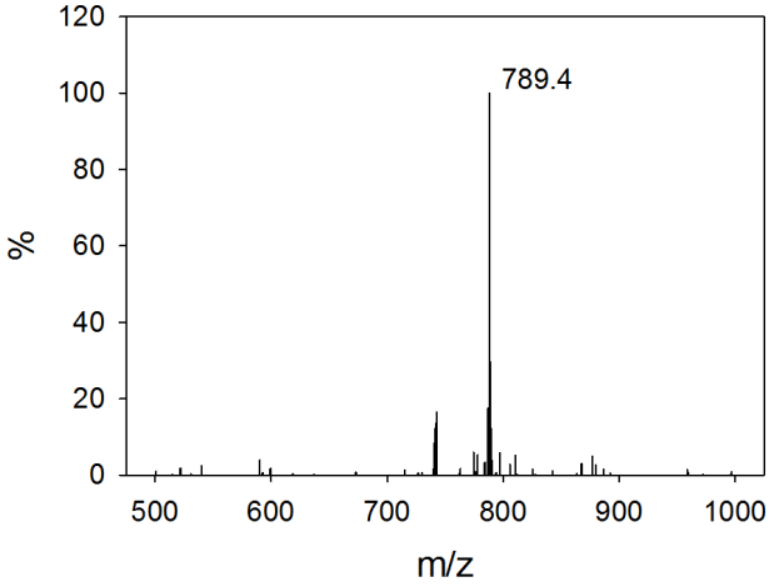


HPLC chromatogram: 1-75 %B, t_R=27.8´

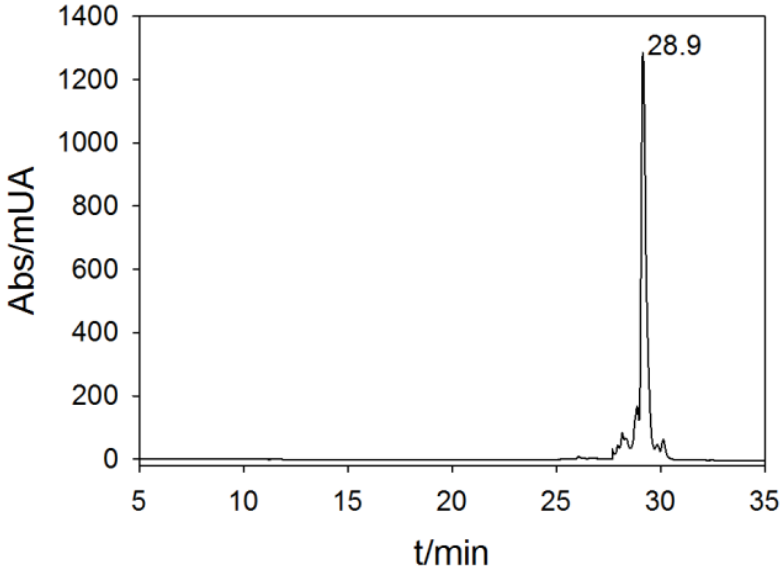


Ir₃

MS(ESI) (m/z): calculated ($[M]^{3+}$, $[C_{110}H_{89}Ir_3N_{19}O_7]^{3+}$): 788.9; found: 789.4.

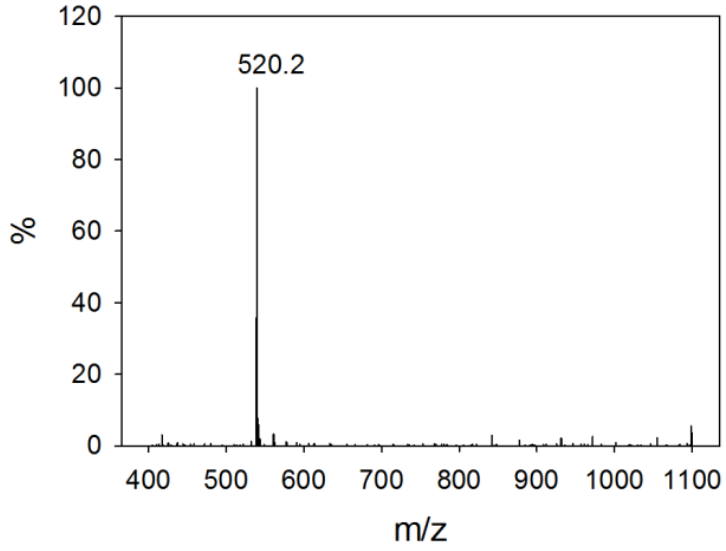


HPLC chromatogram: 1-75 %B, t_R=28.9´

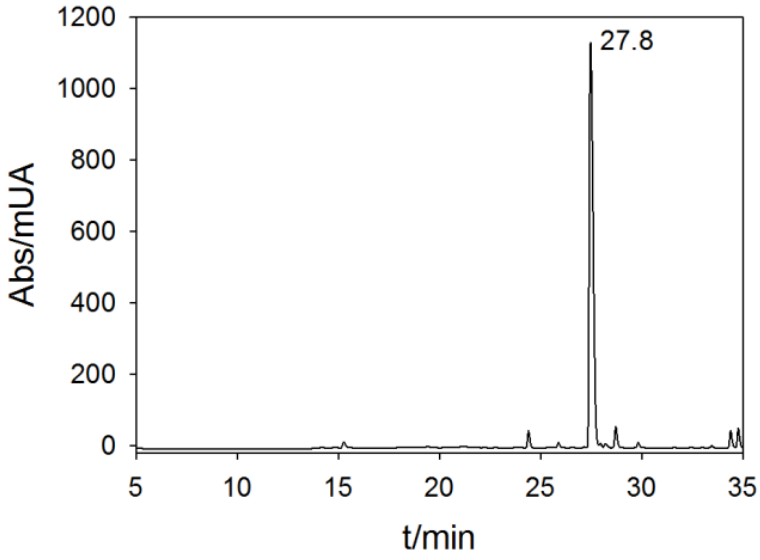


Ir-R₈

MS(ESI) (m/z): calculated ($[M+3H]^{4+}$, $[C_{86}H_{132}IrN_{39}O_{11}]^{4+}$): 520.0, found: 520.2.

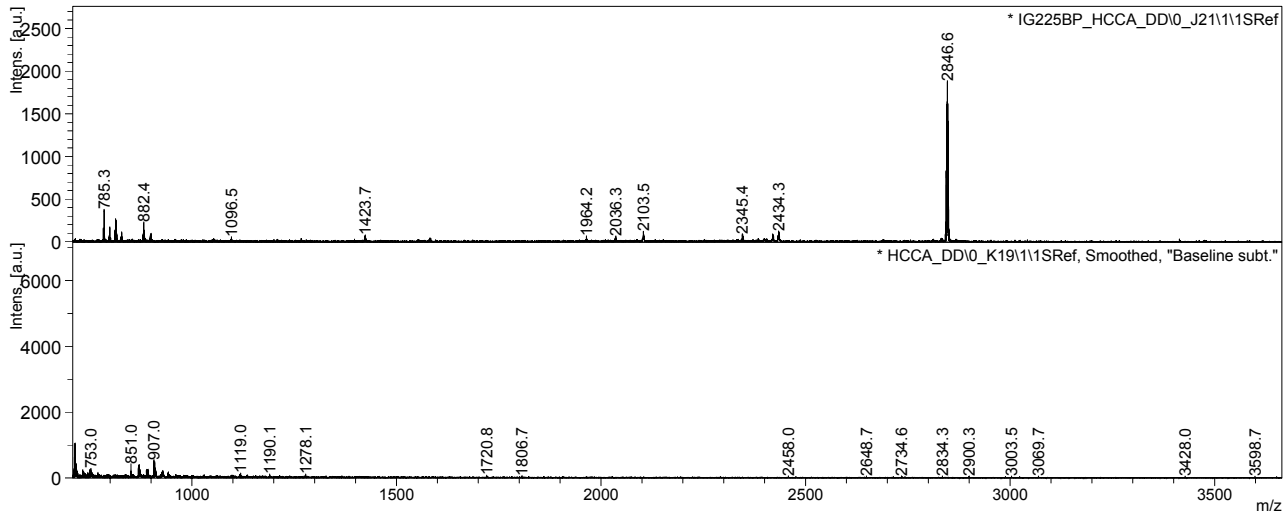


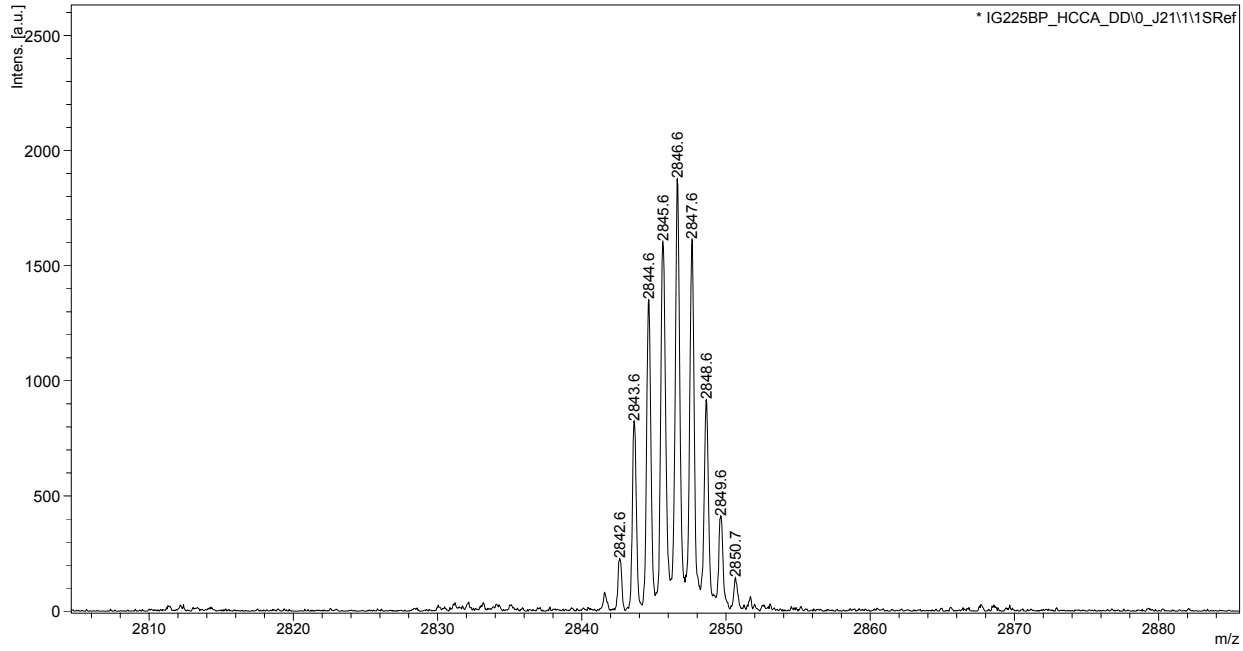
HPLC chromatogram: 1-75 %B, t_R=27.8´



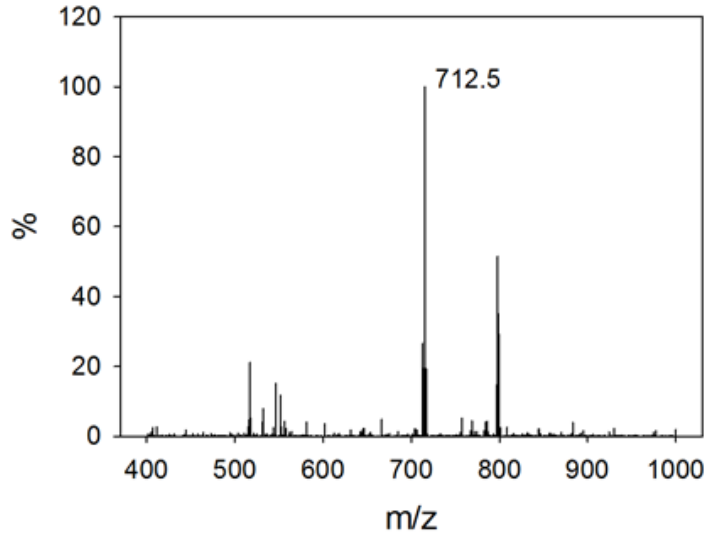
Ir_2-R_8

MALDI-TOF: m/z calculated for $C_{122}H_{157}Ir_2N_{45}O_{13}$: 2846.23, found: 2846.6

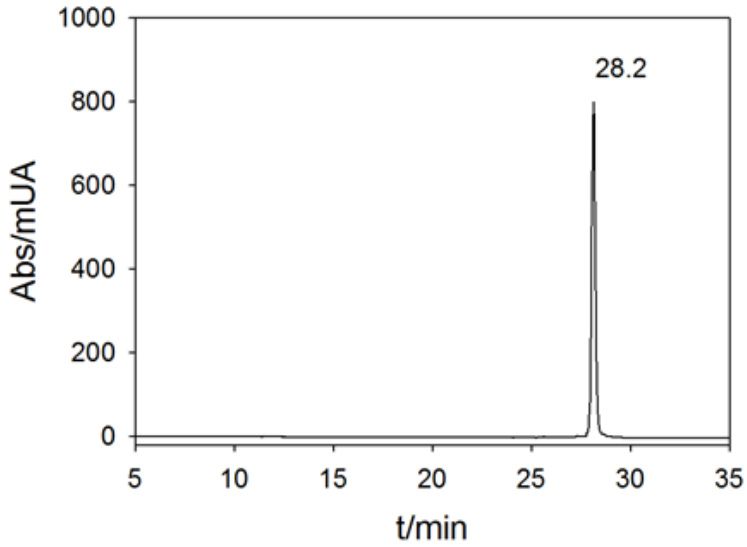




MS(ESI) (m/z): calculated ($[M+2H]^{4+}$, $[C_{122}H_{159}Ir_2N_{45}O_{13}]^{4+}$): 712.1, found: 712.5.

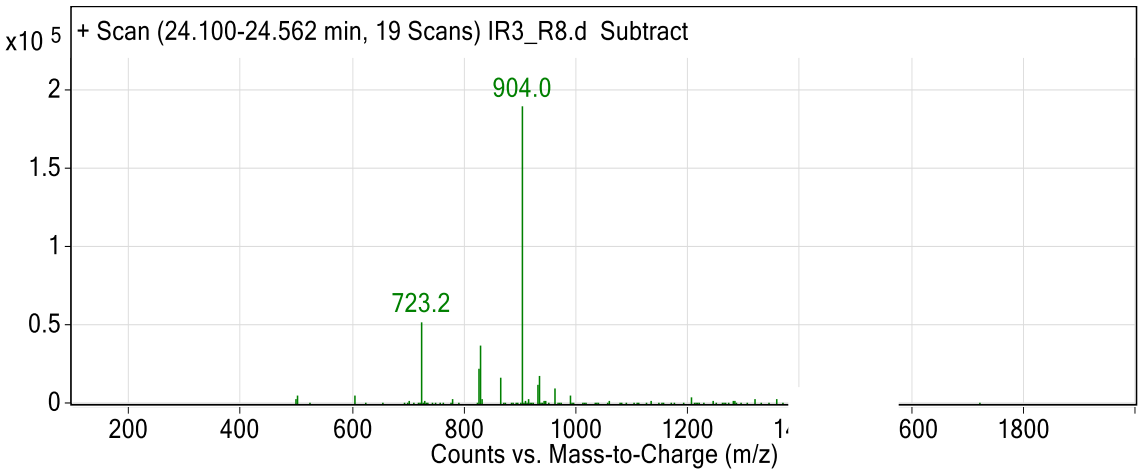


HPLC chromatogram: 1-75 %B, t_R=28.2´

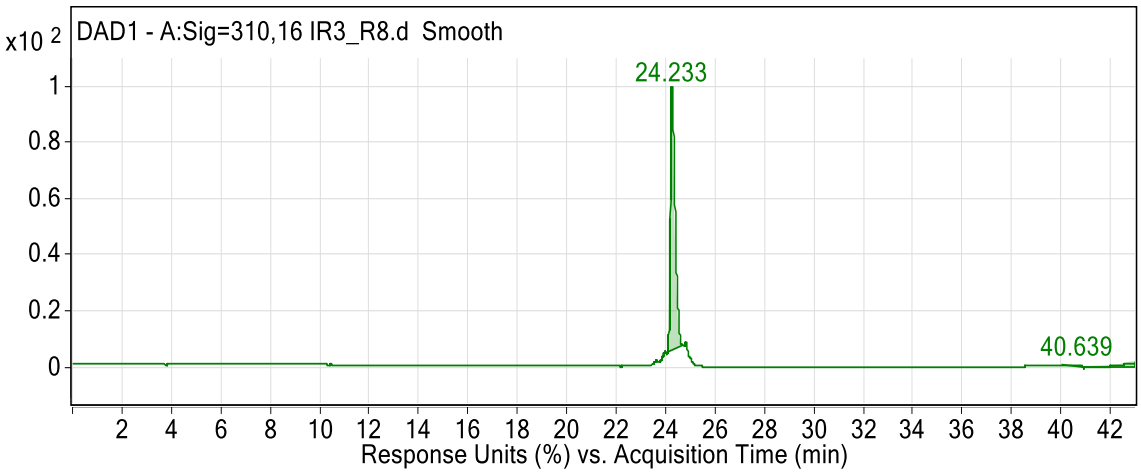


Ir_3-R_8

MS(ESI) (m/z): calculated ($[M+H]^{4+}$, $[C_{158}H_{186}Ir_3N_{51}O_{15}]^{4+}$): 904.1, found: 904.0; calculated ($[M+2H]^{5+}$, $[C_{158}H_{187}Ir_3N_{51}O_{15}]^{5+}$): 723.4, found: 723.2

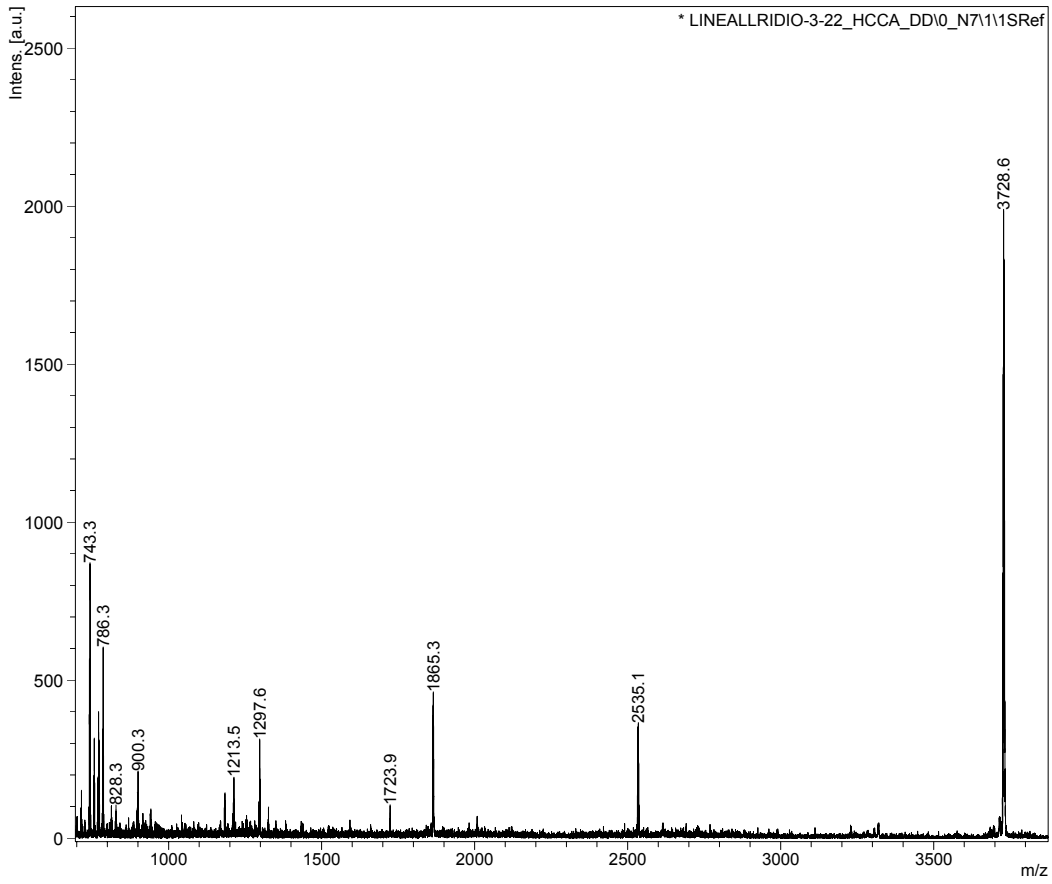


HPLC chromatogram: 1-75 %B, t_R=29.0´

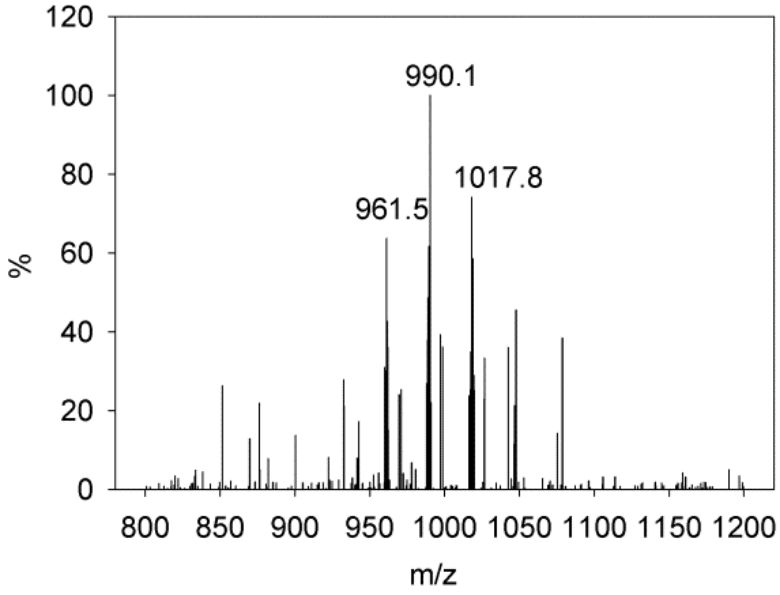


Ir-R$_3$-Ir-R$_3$-Ir-R$_3$

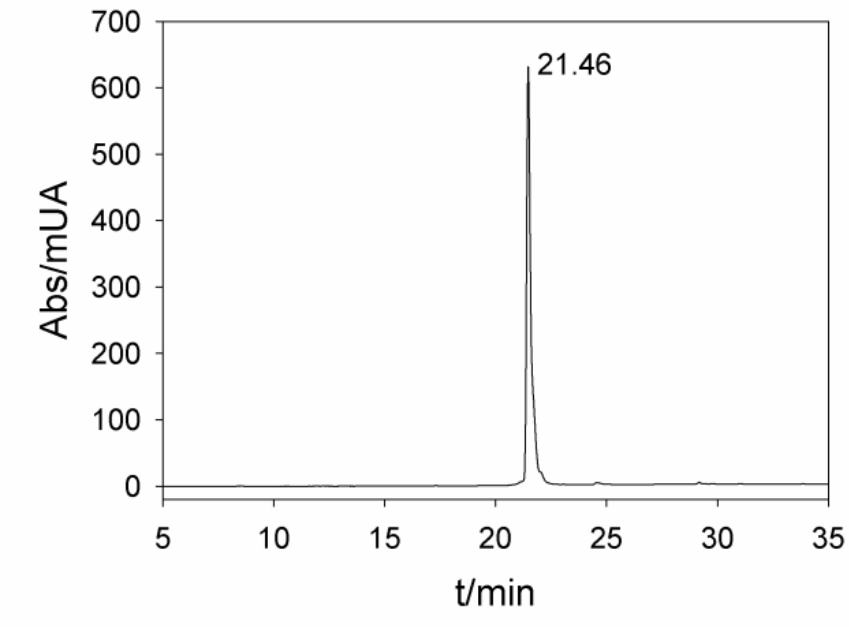
MALDI-TOF: m/z calculated for $C_{162}H_{195}Ir_3N_{55}O_{15}$: 3729.5, found :3728.6



MS(ESI) (m/z): calculated ($[M+H+TFAH]^{4+}$, $[C_{164}F_3H_{197}Ir_3N_{55}O_{17}]^{4+}$): 961.1, found: 961.5; calculated ($[M+H+2TFAH]^{4+}$, $[C_{166}F_6H_{198}Ir_3N_{55}O_{19}]^{4+}$): 989.6, found: 990.1; calculated ($[M+H+3TFAH]^{4+}$, $[C_{168}F_9H_{199}Ir_3N_{55}O_{21}]^{4+}$): 1018.1, found: 1017.8.



HPLC chromatogram: 1-75 %B, t_R=21.4′



Atomic force microscopy (AFM) studies

The AFM images were obtained with a Multimode 8 AFM with electronic Nanoscope V scanning probe microscope from Bruker AXS, using the PEAK FORCE tapping mode. Commercial Si-tip on Nitride lever cantilevers (SNL, Bruker) with force constant of 0.4 Nm^{-1} were used. The samples were deposited on mica disks (PELCO Mica Discs, 9.9 mm diameter; Ted Pella, Inc.), and dried before visualization. Solutions of the metal complexes and the buffer were prepared just prior to use, and filtered through 0.2 nm FP030/3 filters (Scheicher and Schuell, Germany). The samples were prepared in HEPES with relaxed plasmid pBR322 DNA (10 μM in base pairs) and the corresponding metallopeptide ([c] = 25 μM). All samples were incubated for 24 h at 37 ºC before the AFM studies.

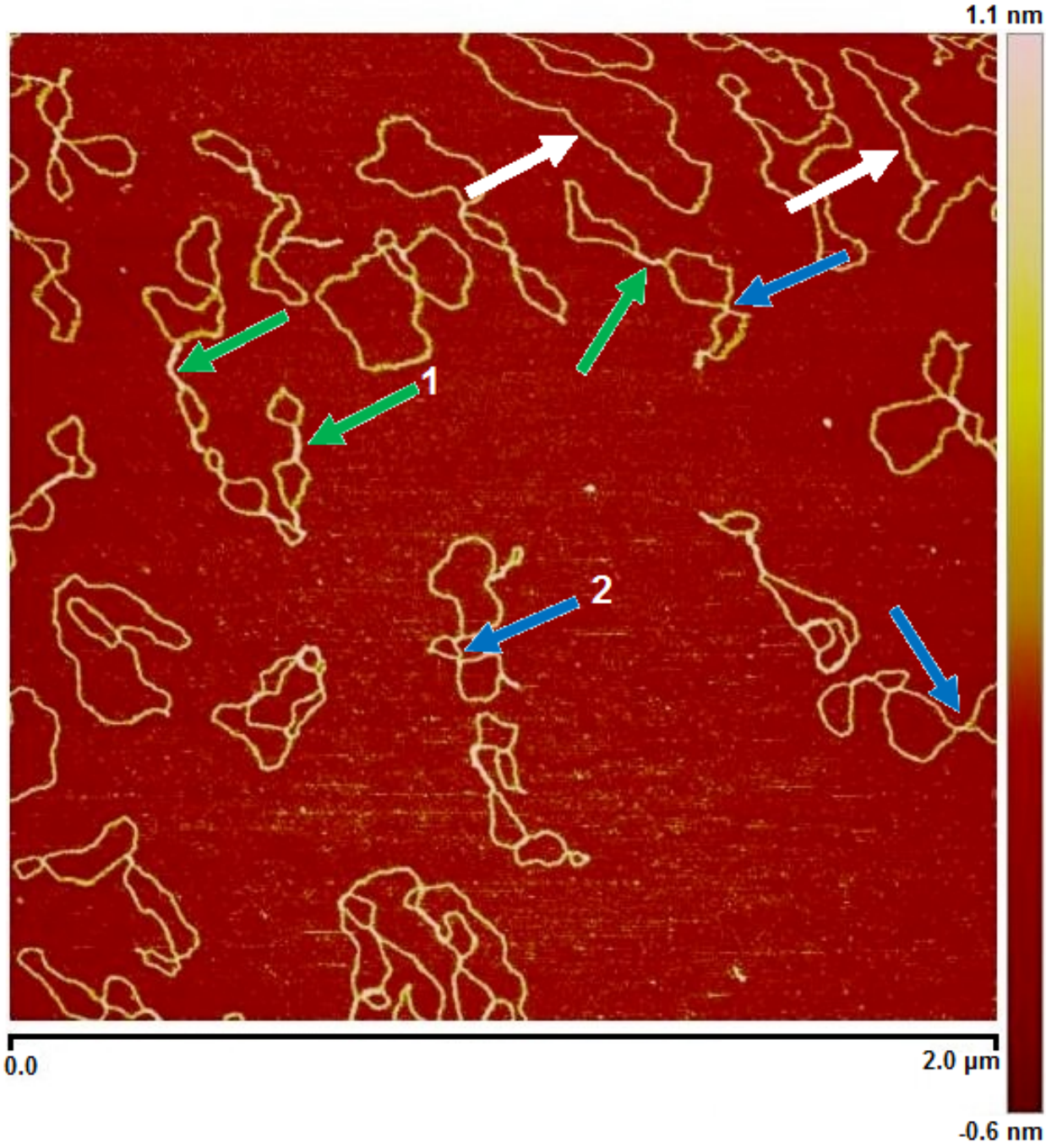


Figure S1. AFM image of pBR322 DNA illustrating the presence of circular forms (white arrows), crossing points (blue arrows) characterizing the initiation of supercoiling, and small supercoiled fragments (green arrows). 1: typical small fragment of supercoiled DNA; 2: example of crossing point from which supercoiling is initiated.

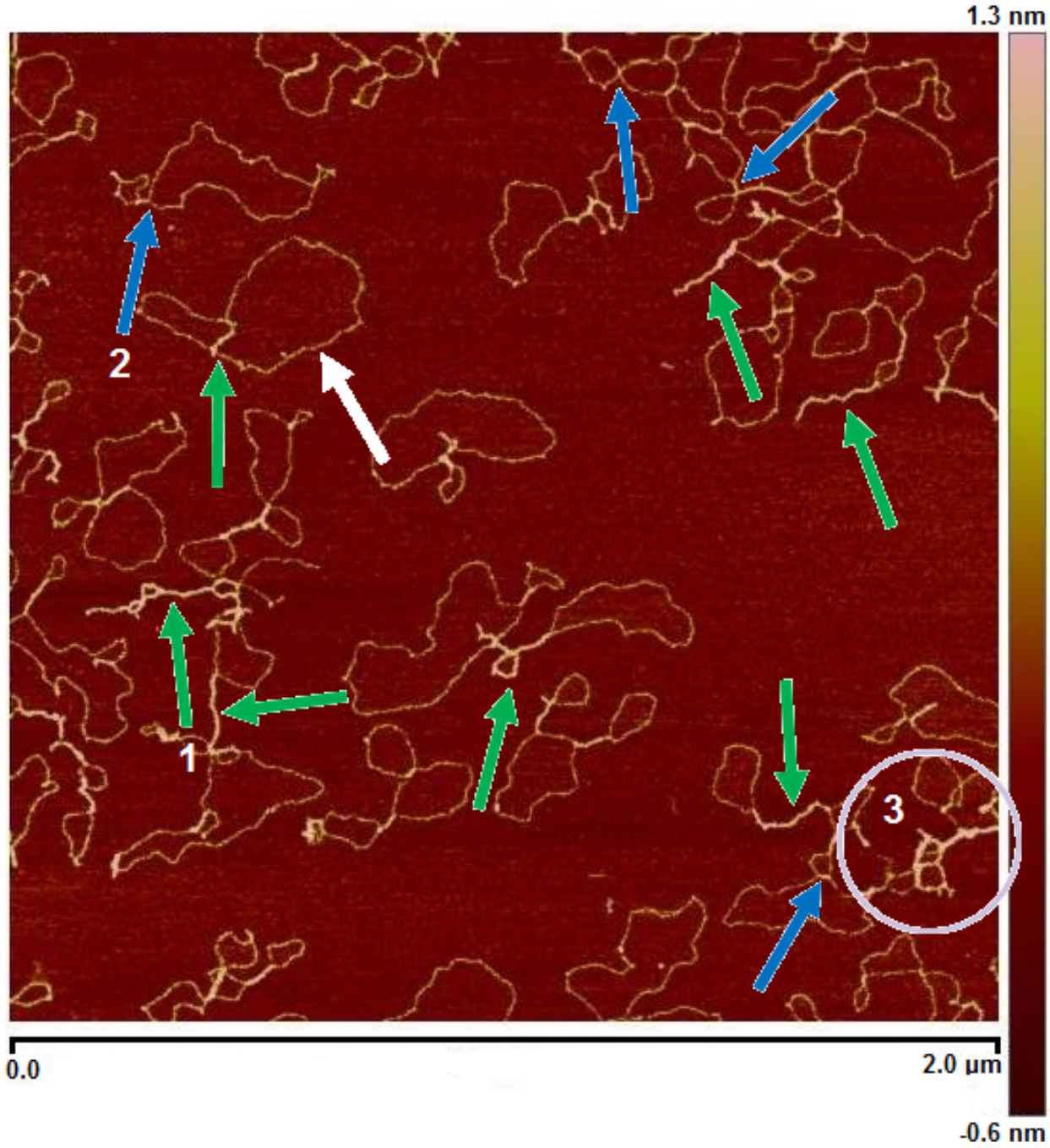


Figure S2. AFM image of pBR322 DNA after 24 h incubation with $\mathbf{Ir_2}$ revealing the presence of circular forms that are partly supercoiled (white arrow), numerous crossing points (blue arrows), very long supercoiled fragments (green arrows), and the formation of DNA aggregates. 1: example of long fragment of supercoiled DNA; 2: crossing point initiating the "closing" of the open circular forms through supercoiling; 3: complex-mediated aggregation of plasmid molecules (*i.e.* various plasmid molecules are gathering through interaction with the metal complex).

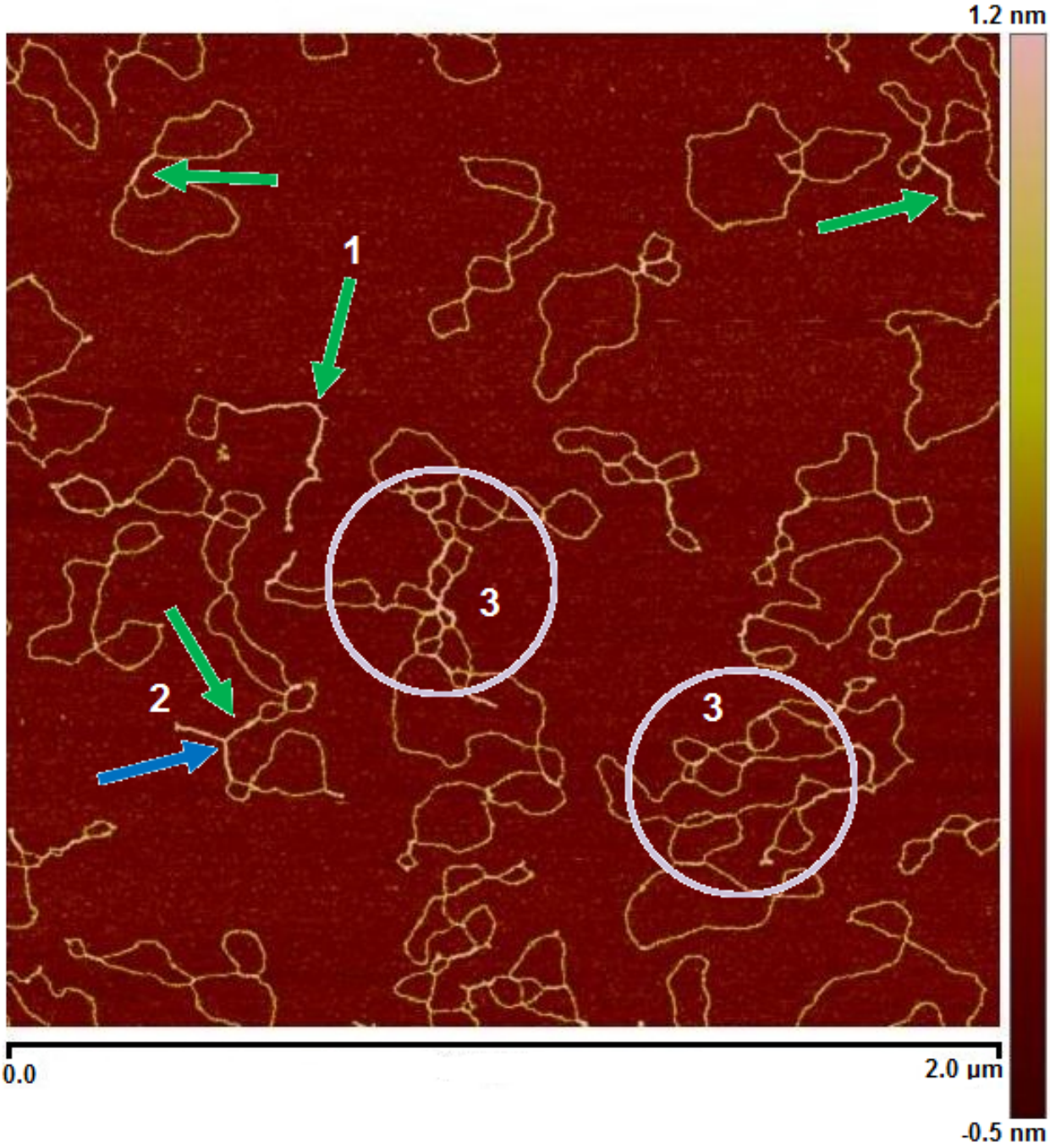


Figure S3. AFM image of pBR322 DNA after 24 h incubation with $\mathbf{Ir_2\text{-}R_8}$ showing the presence of crossing points (blue arrows), very long supercoiled fragments (green arrows), and the formation of large aggregates (grey circles). 1: example of an almost completely supercoiled DNA plasmid; 2: crossing point that initiated supercoiling toward various directions; 3: formation of complex-mediated, large aggregates involving more than two DNA molecules.

Spectrofluorimetric studies

To a 0,2 μL solution of the selected iridium(III) organometallopeptide (**Ir**, **Ir_2, Ir_3, Ir-R_8, Ir_2-R_8, Ir_3-R_8**, **Ir-R_3-Ir-R_3-Ir-R_3**) in phosphate buffer (100 mM), pH 6.8 and NaCl (100 mM), aliquots of dsDNA stock solution (10 μM, in water) were added and the fluorescence spectrum was recorded after each addition. The additions were carried out until no further changes in the emission spectra were detected. The studied oligonucleotides are listed in Table S1.

Table S1. Double and single strand DNA sequences studied.

	Complete sequence (5' to 3')
AAAATT	5'-GGC **AAA ATT** TCG TTTTT CGA AAT TTT GCC-3'
AAGCTT	5'-GGC **AAG CTT** CGC TTTTT GCG AAG CTT GCC-3'
GAAGGC	5'-GGC **GAA GGC** AGC TTTTT GCT GCC TTC GCC-3'
GGCCC	5'-GGC A**GG CCC** AGC TTTTT GCT GGG CCT GCC-3'

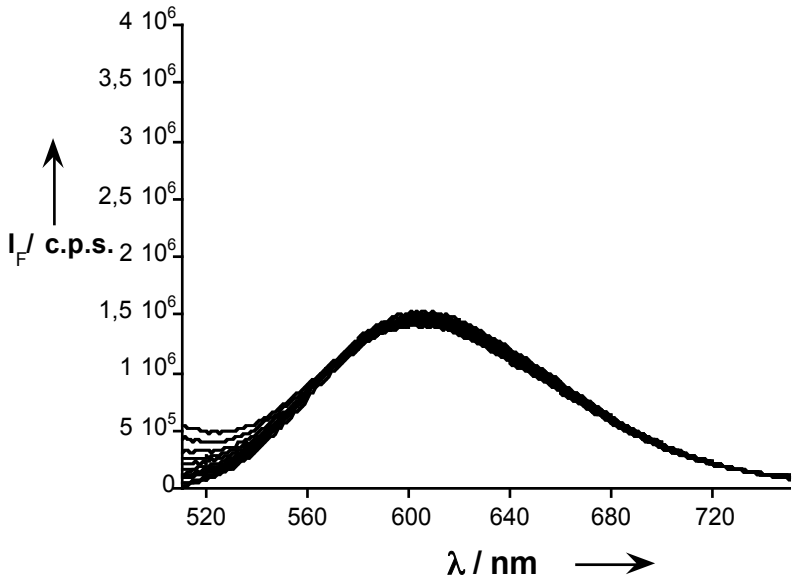


Figure S4. Luminescence spectra of 0.2 μM solutions of **Ir-R_3-Ir-R_3-Ir-R_3** in phosphate buffer (100 mM), NaCl (100 mM), pH 6.8 (red line) and evolution upon addition of aliquots of a GGCCC hairpin oligonucleotide solution (10 μM) until saturation.

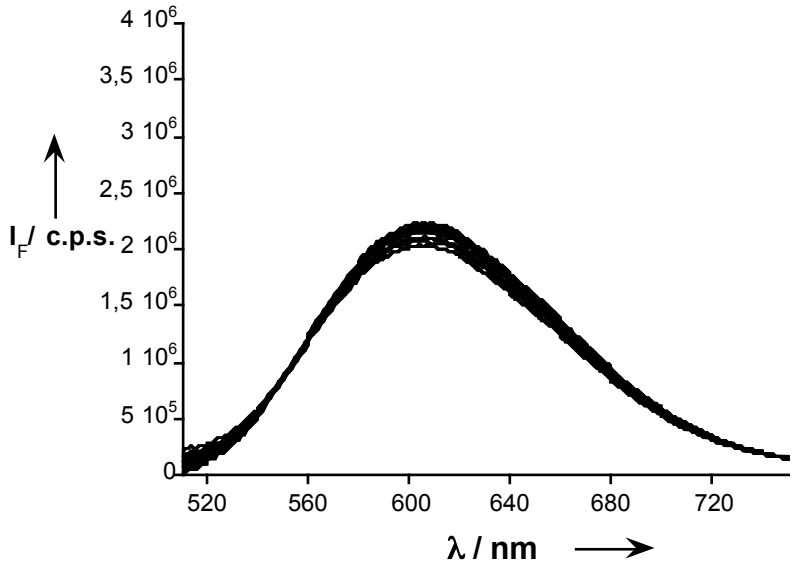


Figure S5. Luminescence spectra of 0.2 μM solutions of **Ir-R_3-Ir-R_3-Ir-R_3** in phosphate buffer (100 mM), NaCl (100 mM), pH 6.8 (red line) and evolution upon addition of aliquots of a AAAATT hairpin oligonucleotide solution (10 μM) until saturation.

Cytotoxicity studies (MTT assay)

General method: Cell lines and grown conditions

Human Breast carcinoma MCF-7, non-small lung carcinoma NCI-H460, cisplatin-resistant ovarian carcinoma A2780cis and doxorubicin-resistance human mamma carcinoma NCI/ADR-RES cell lines, as well as Eagle's Minimal Essential Medium (EMEM) and RPMI1640 were purchased from the American Tissue Culture Collection (ATCC). Frozen MCF-7 cells were thawed in a 75cm^2 cell culture flask in EMEM supplemented with 2-mM L-glutamine, 1.5 g/L Na_2HCO_3, 0.1 mM non-essential aminoacids, 1 mM sodium pyruvate, 0.01 mg/ml bovine insuline and 10% FBS and maintained at 37ºC in a 5% CO_2 atmosphere, replacing medium twice a week. Frozen NCI-H-460 and A2780cis cells were thawed in a 75cm^2 cell culture flask in RPMI supplemented with 2-mM L-glutamine and 10% FBS and maintained at 37ºC in a 5% CO_2 atmosphere, replacing medium twice a week. Frozen NCI/ADR-RES cells were thawed in a 75cm^2 cell culture flask in RPMI 1640 supplemented with 2-mM L-glutamine and 10% FBS and maintained at 37ºC in a 5% CO_2 atmosphere, replacing medium twice a week.

Cytotoxicity assay

The inhibition of cell proliferation induced by the samples was carried out by using MTT method. Cells were seeded in a 96-well microplate (10000, 15000 and 4000 cells/well for MCF-7, NCI-H-460 and A2780cis, respectively) in 100 µl of growth medium and maintained at 37 ºC in a 5% CO_2 atmosphere during 24 hours. Then growth medium was replaced by fresh medium containing different concentrations of the samples to be assayed and maintained at 37 ºC in a 5% CO_2 atmosphere for different times (96 hours for MCF-7 and A2780cis, 48 hours for NCI-H-460 cells, 48 hours for NCI/ADR-RES cells). After this time, 10 µL MTT (5 mg/ml in PBS) were added to each well and maintained for 4 hours at 37 ºC in a 5% CO_2 atmosphere. The 100 µL of 10% SDS in 0.01 M HCl were added to each well an incubated for 12-14 hours under the same experimental conditions. Absorbance due to formazan formation was detected in a Tecan Ultra Evolution reader using a wavelength of 595 nm. All assays were performed with triplicate points.

Data analysis

Data were expressed as the growth inhibition percentage calculated in basis on the equation: % inhibition= 100–[(AO × 100)/AT]. Where AT is the measured absorbance in wells containing compounds and AO is the absorbance measured in blank wells (cells with medium and vehicle). The inhibitory potency of compounds was calculated by constructing concentration-% growth inhibition curves, and extrapolating IC_{50} values (concentration of compound that inhibits cells growth in a 50 %) from these curves. Curves were constructed by using *GraphPad Prism* software V2.01 (GraphPad Inc.).

References

1 I. Gamba, I. Salvadó, G. Rama, M. Bertazzon, M. I. Sánchez, V. M. Sánchez-Pedregal, J. Martínez-Costas, R. F. Brissos, P. Gamez, J. L. Mascareñas, M. Vázquez López, M. E. Vázquez, *Chem. Eur. J.*, **2013**, *19*, 13369–13375.

2 S. Sprouse, K. A. King, P. J. Spellane, R. J. Watts, *J. Am. Chem. Soc*., **1984**, *106*, 6647–6653.