Supplementary Information

Cytotoxicity and Immunogenic Cell Death Studies of Water-soluble Tetrahedral Ga(III) or Fe(III) Coordination Cages Containing a Gold(I) Anticancer Drug as Guest

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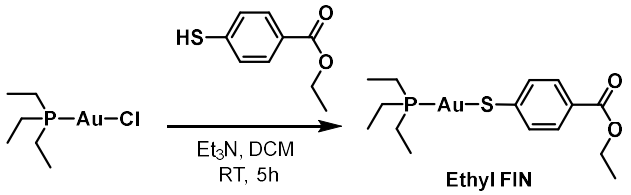
1. Experimental and Methods Section

1.1 Instrumentation. A Varian Inova 500 MHz NMR spectrometer (11.7 T) equipped with FTS Systems TC-84 Kinetics Air Jet Temperature Controller or a Bruker Neo 500 MHz spectrometer was used to collect ^{1}H NMR spectra. Water ^{1}H relaxivity experiments were performed at 1.4 T (34 ^{o}C) on a Nananalysis NMR spectrometer. All pH measurements were made by utilizing an Orion 8115BNUWP Ross Ultra Semi Micro pH electrode connected to a 702 SM Titrino pH. A Thermo Fisher Linear Ion Trap (LTQ) LC/MS equipped with a Surveyor HPLC system was used to collect mass spectrometry data of the complexes. Iron, gallium, and gold concentration was determined by using a Thermo X-Series 2 ICP-MS and UV-vis spectrometry as reported.[1] The FT-IR spectra were recorded on a Vertex 70 FTIR Spectrometer (Bruker) equipped with a ZnSe single-reflection 45° angle ATR accessory (Pike Technologies Inc.).

1.2. Reagents. Solvents and reagents were used without further purification unless otherwise specified. 2,3-dimethoxy-benzoic acid was purchased from TCI. 1,5-diaminonapthalene was purchased from Alfa Aesar. A 1 M boron tribromide solution in dichloromethane was purchased from Sigma-Aldrich. Ga(III) acetylacetonate was purchased from Beantown chemical corporation. Linker H_4A was prepared as reported.[2] The gallium and iron cages were prepared as reported.[3] Triethylphosphine gold(I) chloride was purchased from Strem chemicals Inc., USA. 4-mercaptobenzoic acid was procured from Ambeed. The FIN complex was prepared as reported.[4] Triethylamine was purchased from Merck, USA. Triethylphosphine was purchased from Strem chemicals, USA.

1.3. Synthesis

1.3.1. Synthesis of Ethyl-FIN



Scheme S1. Synthesis of Ethyl-FIN.

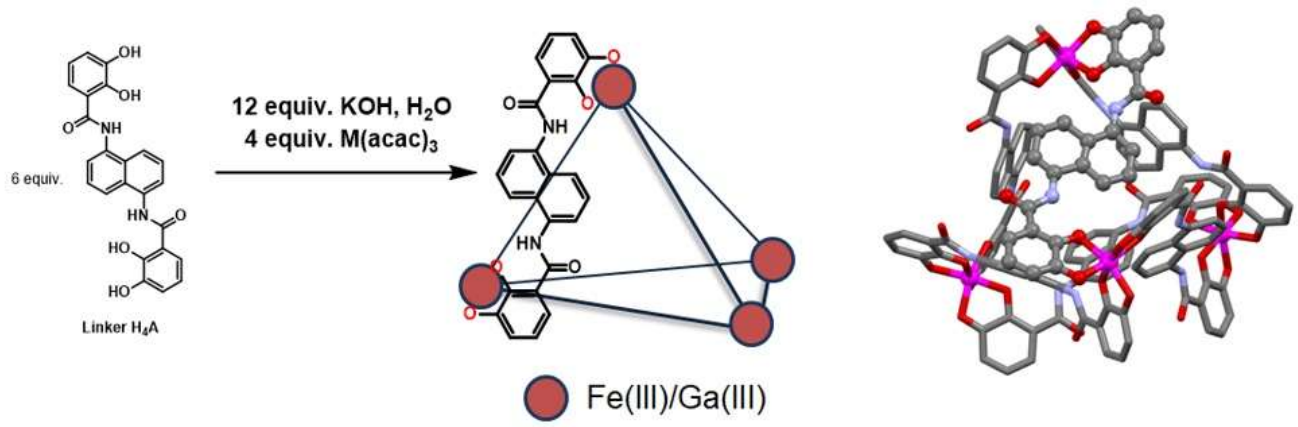
In a 100 mL round bottom flask, ethyl 4-mercaptobenzoate (67.5 mg, 0.37 mmol) was dissolved in 10 mL of dichloromethane and Et_3N (87.4 μL, 0.62 mmol) was added to the reaction solution. Then $Au(PEt_3)Cl$ (100 mg, 0.28 mmol) was added to the reaction mixture and stirred at room temperature for 5 h. The progress of the reaction was monitored using thin layer chromatography. The solvent was evaporated, and the product was purified using column chromatography (silica gel and 20% ethyl

acetate in hexane) to obtain a light yellow solid. Yield 115 mg (81.6%). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm): 7.62 (d, J = 12 Hz, 2H), 7.46 (d, J = 12 Hz, 2H), 4.24 (q, J = 8 Hz, 2H), 1.96 (q, J = 8 Hz, 6H), 1.28 (t, J = 8 Hz, 3H), 1.14 (q, J = 8 Hz, 9H). ^{13}C NMR (100 MHz, DMSO-D_6): δ (ppm): 166.2, 153.0, 131.7, 129.1, 124.4, 60.6, 17.6, 14.6, 9.5. ^{31}P NMR (162 MHz, DMSO-D_6): δ (ppm): 39.5. ESI-MS: $C_{15}H_{24}AuO_2PS$, *m/z* = calculated 496.09 M^+ found 496.08. A sample of Ethyl-FIN was expected to have a content of 197.0 ppb gold. The observed Au content from ICP-MS analysis was obtained as follows: 189.7 $\pm$0.4 ppb of Au, indicating ~96.3 % purity.

1.3.2. Synthesis of Ga-FIN (1a)

Linker H_4A (Scheme S1) (0.100 g, 0.232 mmol) was added to a round-bottom flask. A solution of potassium hydroxide (0.464 mmol) in water (5.0 mL) was added into the reaction flask and stirred. Ga(III) acetylacetonate (0.056 g, 0.154 mmol) was added directly into the reaction mixture, and the mixture was stirred for 2 h at room temperature. Then FIN complex (0.014 g, 0.029 mmol) was added to the reaction mixture and stirred for 1 h to produce a clear solution. The reaction solution was filtered and evaporated to dryness. The resulting solid was dissolved in acetone, warmed for 15 min, and then cooled to 0 °C. The precipitate was isolated by filtration and washed with cold acetone. A purple crystalline product was obtained and dried under a vacuum. Yield: 62%. ^{13}C NMR (125 MHz, D_2O):174.8, 169.7, 168.4, 158.6, 155.4, 154.7, 152.8, 133.8, 131.8, 129.2, 127.2, 125.9, 116.5, 116.0, 115.5, 114.9, 114.4, 17.0, 8.5. δ (ppm) ^{31}P NMR (202 MHz, D_2O): δ(ppm) 44.7. Samples were expected to have a content of 69.00 ppb of Ga and 49.25 ppb of gold. The observed contents from ICP-MS analysis were obtained as follows: 65.6 ± 0.2 ppb of Ga and 46.4 ± 0.3 ppb of Au, indicating ~95% purity based on gallium and a 4:1 ratio of Ga to Au and a formula of K_{12} [**1**(Au(PEt_3)($SC_6H_4CO_2H$)].

Scheme S1. General synthetic reaction for M_4L_6 type tetrahedral cage ($K_{12}[M_4(A_6)]$) M = Ga(III) or Fe(III). Right image shows ball and stick model of cage.[3] The procedure was adapted from literature.[2]



1.3.3. Synthesis of Ga-Ethyl FIN (1b)

Linker H_4A (0.100 g, 0.232 mmol) was added to a round-bottom flask. A solution of potassium hydroxide (0.464 mmol) in water (5.0 mL) was added into the reaction flask and stirred. Ga(III) acetylacetonate (0.056 g, 0.154 mmol) was added directly into the reaction mixture, and the mixture was stirred for 2 h at room temperature. Then Ethyl FIN gold complex (0.0184 g, 0.037 mmol) was added to the reaction mixture and stirred for 1 h to produce a clear solution. The reaction solution was filtered and evaporated to dryness. The resulting solid was dissolved in acetone, warmed for 15 min, and then cooled to 0 °C. The precipitate was isolated by filtration and washed with cold acetone. A purple crystalline product was obtained and dried under a vacuum. Yield: 74%. ^{31}P NMR (202 MHz, D_2O): δ(ppm) 44.8. Samples were expected to have a content of 69.00 ppb of Ga and 49.25 ppb of gold. The observed contents from ICP-MS analysis were obtained as follows: 66.5 ± 0.3 ppb of Ga and 48.4 ± 0.2 ppb of Au, indicating ~96% purity based on gallium and a 4:1 ratio of Ga to Au for a molecular formula of K_{12} [**1(**$Au(PEt_3)(SC_6H_4CO_2C_2H_5)$**]**.

1.3.1. Synthesis of Fe-FIN (2a)

Linker H_4A (0.100 g, 0.232 mmol) was added to a round-bottom flask. A solution of potassium hydroxide (0.464 mmol) in water (5.0 mL) was added to the reaction flask with stirring. Iron(III) acetylacetonate (0.054 g, 0.154 mmol) was added directly into the reaction mixture and stirred for 2 h at room temperature. Then FIN complex (0.014 g, 0.029 mmol) was added to the reaction mixture, and the mixture was stirred for 1 h to produce a clear solution. The reaction solution was filtered and then evaporated to dryness. The solid was dissolved in acetone, warmed for 25 min, and then cooled down to 0 °C. The precipitate was filtered and washed with cold acetone. An orange crystalline product was obtained and dried under vacuum. Yield: 64%. The effective magnetic moment was measured as 5.7 by using the Evans method. Samples were expected to have a content of 56.00 ppb of Fe and 49.25 ppb of gold. The observed contents were obtained as follows: 54.4 ± 0.3 ppb of Fe and 47.4 ± 0.4 ppb of Au, indicating ~97% purity based on iron and a 4:1 ratio of Fe to Au and a molecular formula of K_{12} [**1(**$Au(PEt_3)(SC_6H_4CO_2H)$**]**.

1.3.2. Synthesis of Ga-Au(PEt_3)$_2$ (1c)

Linker H_4A (0.100 g, 0.232 mmol) was added to a round bottom flask. A solution of potassium hydroxide (0.464 mmol) in water (5.0 mL) was added into the reaction flask and stirred. Ga(III) acetylacetonate (0.056 g, 0.154 mmol) was added directly to the reaction mixture and stirred for 2 h at room temperature. Then $[(Et_3P)_2Au]Cl$ (0.016 g, 0.0341 mmol) was added to the reaction mixture and stirred for 1 h to produce a clear solution. The reaction solution was filtered and evaporated to dryness. The resulting solid was dissolved in acetone, warmed for 15 minutes and then cooled to 0 °C. The precipitate was isolated by filtration and washed with cold acetone. A purple crystalline product was obtained and dried under vacuum. Yield: 68%. ^{1}H NMR (500 MHz, D_2O): δ (ppm) 7.95 (d, J = 7.5 Hz, 12H), 7.85 (d, J = 8.5 Hz, 12H), 7.29 (d, J = 7.5 Hz, 12H), 7.09 (t, J = 8.5 Hz, 12H), 6.76 (d, J = 7.5 Hz, 12H), 6.60 (t, J = 7.5 Hz, 12H), -0.67 to -0.18 (m, 18H), -1.36 to -1.61 (m, 12H). ^{31}P NMR (202 MHz, D_2O): δ(ppm) 41.2. Samples were expected to have a content of 69.00 ppb of Ga and

49.25 ppb of gold. The observed contents from ICP-MS analysis were obtained as follows: 64.4 ± 0.3 ppb of Ga and 46.6 ± 0.3 ppb of Au, indicating ~93% purity based on gallium and a 4:1 ratio of Ga to Au and a molecular formula of K_{11} [**1(**$Au(PEt_3)_2$**]**.

1.4. Experimental details for mass spectrometry studies

A Thermo Fisher Linear Ion Trap (LTQ) LC/MS equipped with a Surveyor HPLC system was used to study the release of the gold complex from the coordination cages as a function of pH. One equivalent of **1a**, Ga-FIN (4.0×10^{-4} M) was dissolved in 0.8 mL of Mili-Q water, and the pH of the solution was adjusted as desired with 0.1 M HCl or 0.1 M NaOH. These solutions were then mixed with a solution containing one equivalent of Bu_4NPF_6 in 0.4 mL of methanol. The final solution was filtered through a 0.45 μm sterile syringe filter, and 0.005 mL was injected into the mass spectrometer for analysis. A similar procedure was followed while analyzing the release of the gold complex from a solution of **1b (**Ga-EthylFIN).

1.5 Stability of the coordination cages at acidic conditions and with ascorbate

FIN encapsulated in gallium cage **1a**, Ga-FIN (1 mM) was prepared in D_2O and/or 1X PBS buffer (pH 7.4) and treated with one equivalent of ascorbic acid (1 mM). The resulting solution was incubated for 1h at 37 ºC and the corresponding ^{1}H NMR spectrum was recorded. Alternatively, solutions of **1a**, Ga-FIN (10-50 μM) or 2a, Fe-FIN (5-50 μM), 10 mL were prepared in 0.1 M NaCl and treated with one equivalent of ascorbic acid. The pH of the solution was adjusted with 0.1 M HCl or 0.1 M NaOH. Electronic absorption spectra were recorded.

1.6 Cell Viability Studies

1.6.1 Cell Culture

Human clear cell renal cell carcinoma (Caki-1) and murine CT26 (derived from a mouse with N-nitroso-N-methylrethane induced colon carcinoma) cell lines were cultured using Roswell Park Memorial Institute (RPMI-1640; Fisher Scientific, Hampton, NH, USA) and supplemented with 10% FBS, 1% MEM-NEAA, and 1% PenStrep. IMR-90 human lung fibroblast cells were cultured with Dulbecco's modified Eagle's medium (Fisher Scientific) containing 10% fetal bovine serum (FBS; Fisher Scientific), 1% penicillin–streptomycin (PenStrep; Fisher Scientific), and 1% minimum essential medium (MEM) nonessential amino acids (NEAA; Fisher Scientific). All cell lines were obtained from the American Type Culture Collection (Manassas, Virginia, USA) and cultured in a humidified incubator at 37 °C under 5% CO2 and 95% air.

1.6.2 Cell viability

The cytotoxicity profiles of the Ga(III) and Fe(III) cages (**1** and **2**), Ga-FIN (**1a**) and Fe-FIN (**2a**) in Caki-1, CT26 and IMR90 cell lines at various pH levels were assessed using the colorimetric cell

viability assay PrestoBlue (Thermo Fisher Scientific). Cells were seeded in 96-well plates at 5.6×10^3 – 6×10^3 cells per well in 100 µL of their respective complete growth media and incubated at 37 °C with 5% CO_2 for 24 hours to allow attachment. The cage and metal-cage complexes were dissolved in distilled H_2O and diluted with the appropriate media to achieve concentrations ranging from 10–150 µM. Auranofin and FIN were dissolved in 0.01% DMSO (and diluted with media). Media at pH 7.5, 7.2 (control), 6.5, and 6.2 were prepared by titration with HCl or NaOH. After 24 or 72 hours of incubation with the compounds at the designated pH levels, the culture medium was replaced with fresh medium containing 11 µL of Presto-Blue solution. Cells were incubated for an additional hour at 37°C and 5% CO_2. The optical fluorescence was measured at 560/590 nm on a BioTek Synergy Multi-mode microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. Controls consisted of untreated cells at each pH level to ensure that any observed changes in viability were due to compound activity and not pH-induced effects. IC_{50} values were fit using GraphPad Prism 7 and the appropriate curve. Data are presented as mean ± SEM from at least three independent experiments, each with triplicate measurements.

1.5. ^{69}Ga and ^{197}Au quantification in cell lysates (ICP-MS)

A Thermo X-Series 2 ICP-MS was used for the determination of Ga and Au concentrations. Cells were seeded in 6-well plates at 1×10^5 cells per well, and the corresponding volume (approximately 0.6-0.8 mL) was transferred into the sample tubes. Stock solutions of cell lysates were diluted to 1.00 mM sample solutions. 100.0 µL aliquots of the sample solution were added to 900.0 µL of 70% (v/v) metal-free HNO_3. Samples were allowed to digest for 3 days, after which they were diluted to 2% HNO_3 at approximately 69 ppb Ga in 10.0 mL of Milli-Q water. Solutions of gallium and gold samples ranging from 0.1 to 100 ppb were prepared for the quantification by a linear calibration curve. Indium and rhodium standards were used as internal standards in the case of gallium and gold, respectively. Data analysis was performed by using PlasmaLab software.

1.6. Immunogenic Cell Death Studies

1.6.1. Extracellular ATP concentration detection

Caki-1 cells were seeded at a cell density of 100,000 cells/mL in a 12-well plate and grown in RPMI-1640 media with 10% FBS in a cell incubator with a 5% CO2 at 37ºC for 24 h. The cells were then incubated with the desired compounds in complete RPMI at their respective IC50 values for 24 h in the incubator. The extracellular ATP concentration was measured using the luciferase luminescence assay- InvitrogenTM ATP determination kit (A22066).

1.6.2. Intracellular HMGB-1 detection

Caki-1 cells were seeded at a cell density of 100,000cells/mL onto a 12-well plate and were grown in RPMI-1640 media with 10% FBS (complete RPMI) in the cell incubator with 5% CO2 atmosphere at 37ºC for 24 h. The cells were then incubated with compounds in complete RPMI for 24 h at 37ºC. HMGB1 release into cell culture supernatants was quantified using a commercially available human

HMGB1 ELISA kit (EEL047) following the manufacturer's instructions. After 24-hour treatment, supernatants were collected, centrifuged to remove cellular debris, and incubated in pre-coated ELISA plates. Absorbance was measured at 450 nm using a plate reader. The protein concentrations were calculated from a standard curve. All experiments were conducted in 3 independent biological replicates.

1.6.3. CRT detection

Caki-1 cells were plated overnight into a 4-well Nunc™ Lab-Tek™ II Chambered Coverglass (155382PK) at 1.5 x 104 cells/ well (1ml per chamber). Cells are treated with the IC50 concentrations of the complexes for 24h in a cell incubator at 37 °C in a 5% CO2 atmosphere. After treatment, cells were washed with 1ml of PBS then fixed in 4% paraformaldehyde in PBS for 20 min at room temperature. Wash cells twice with 1 ml PBS and add the CRT ab conjugated with Alexa Fluor 647 (1:500) overnight at 4°C. Wash twice with PBS and incubate with 5µg/mL Wheat Germ Agglutinin (WGA) conjugated to Alexa Fluor 488 (W11261, Invitrogen) at room temperature for 30 minutes. Wash with PBS and add DAPI mounting solution, and place on the coverslip. Images were acquired using the Laser Confocal Scanning Microscope (FV10i).

Spectroscopic characterization of Ethyl-FIN and Au-containing Ga and Fe (cages) including release and stability experiments.

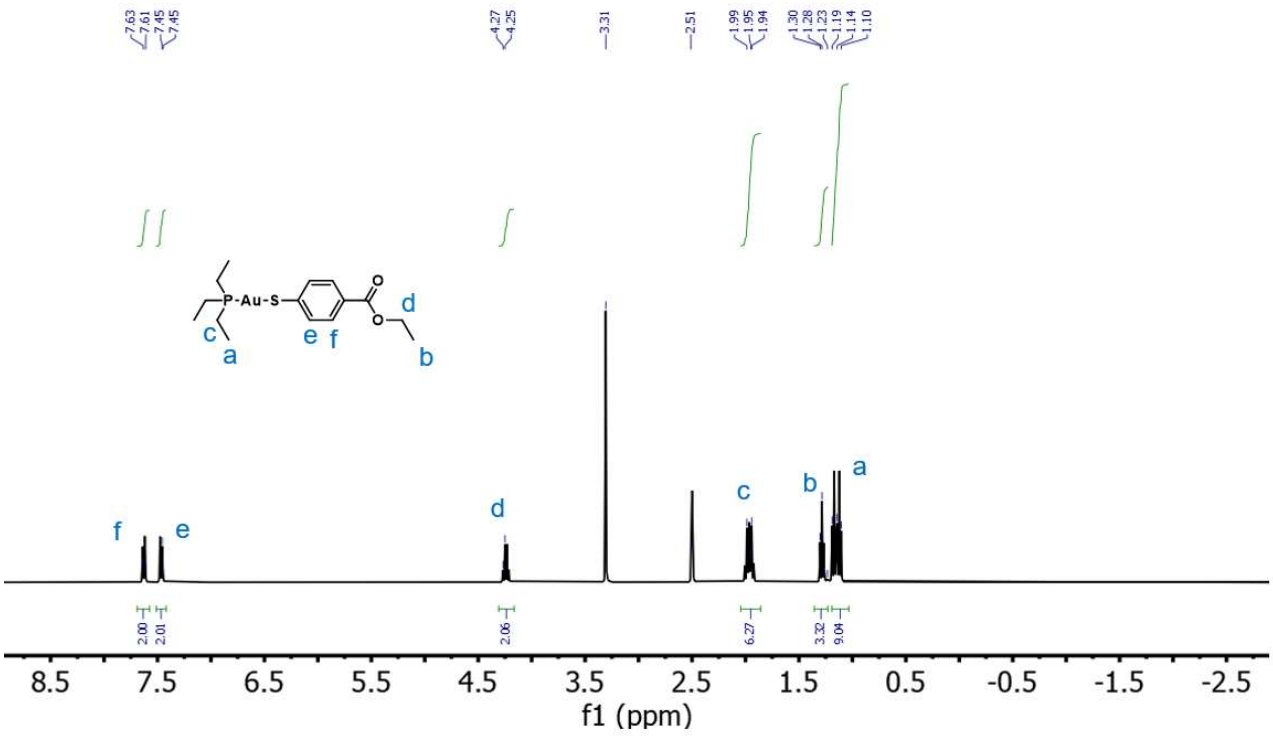


Figure S1. ^{1}H NMR spectrum (400 MHz) of ethyl-FIN in DMSO-D_6.

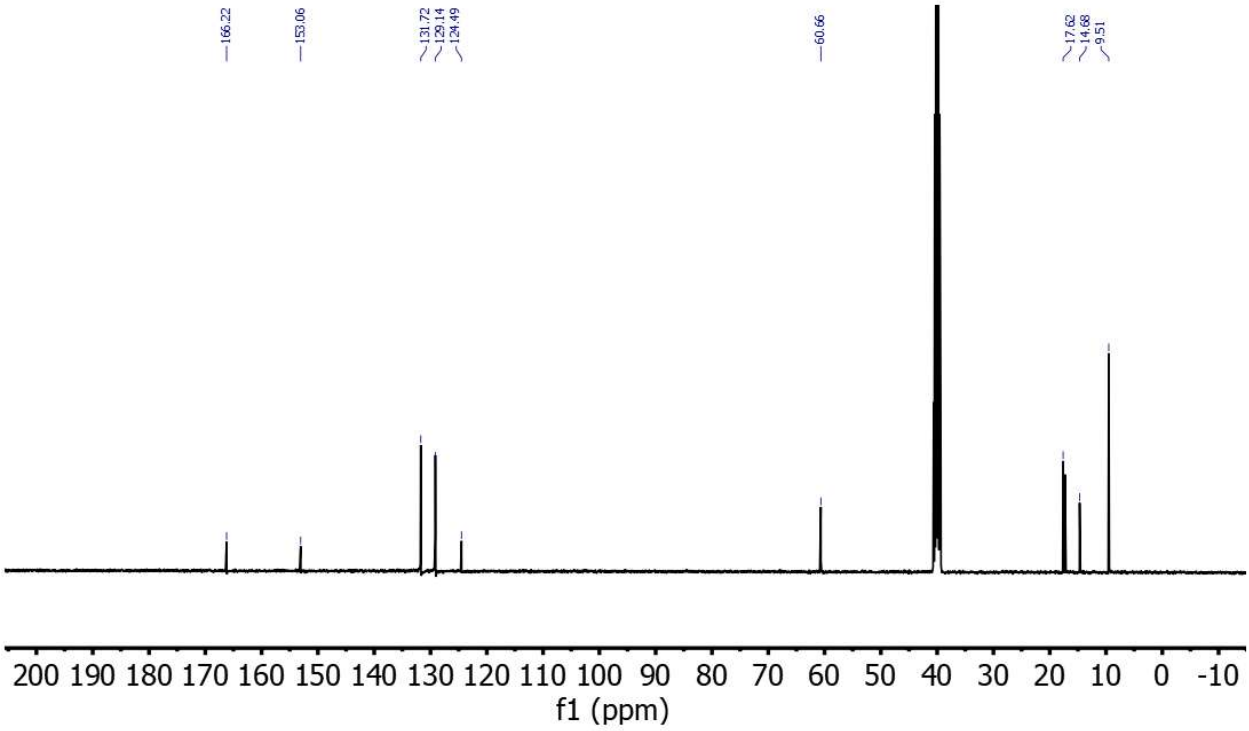


Figure S2. $^{13}C\{^{1}H\}$ NMR spectrum (100 MHz) of ethyl FIN in DMSO-D_6.

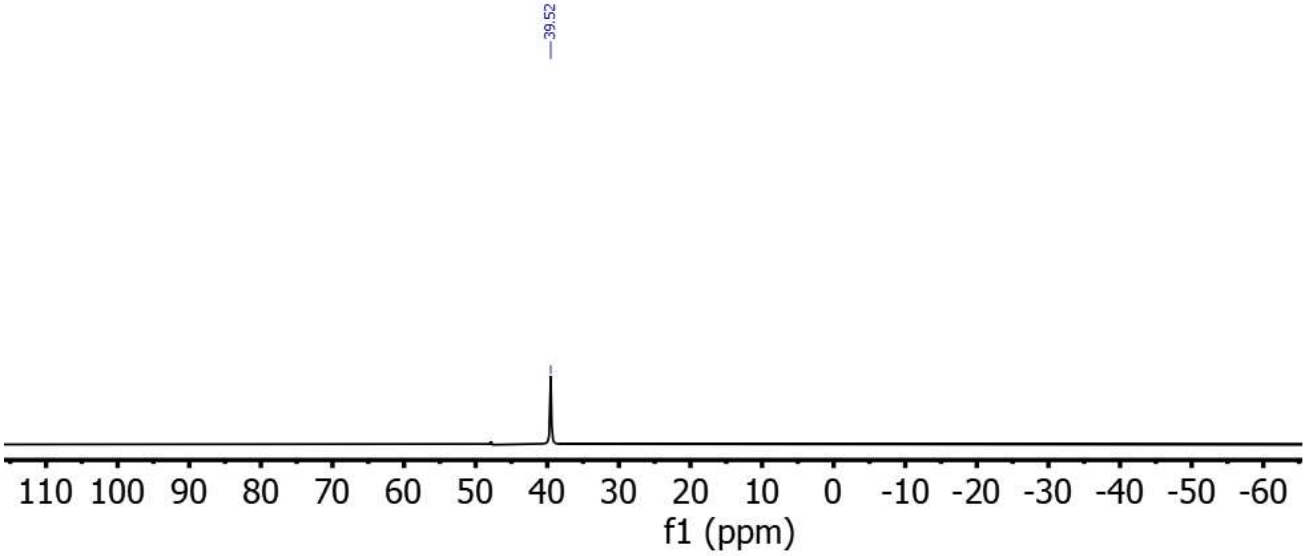


Figure S3. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz) of ethyl FIN in DMSO-D_6.

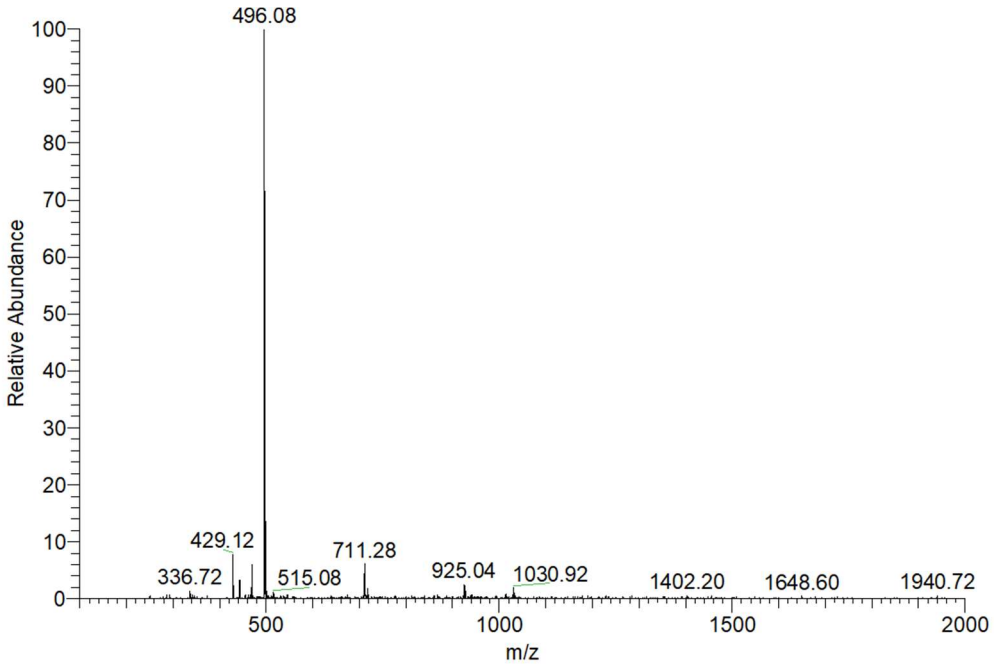


Figure S4. ESI-MS spectrum of ethyl FIN complex.

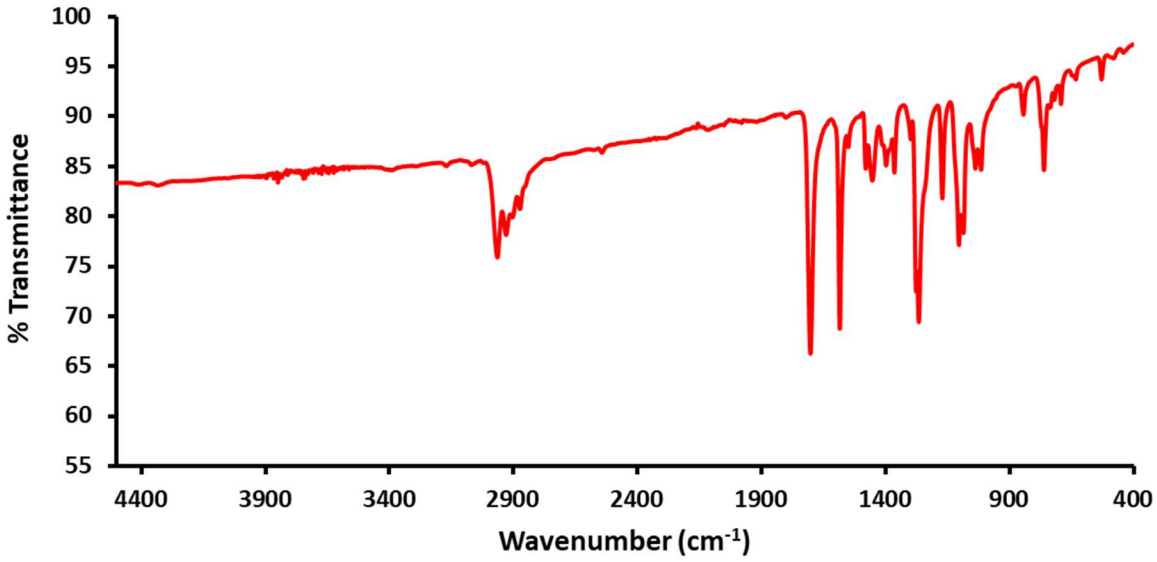


Figure S5. FT-IR spectrum of ethyl FIN complex.

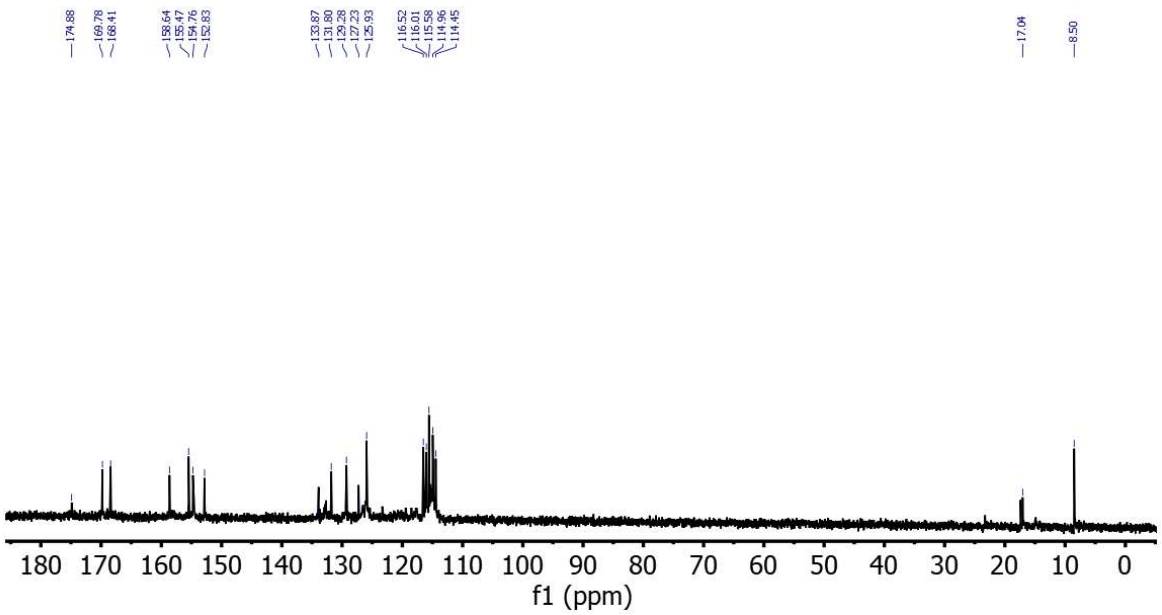


Figure S6. $^{13}C\{^{1}H\}$ NMR spectrum (125 MHz) of Ga-FIN (**1a**) (FIN encapsulated Ga cage) in D_2O.

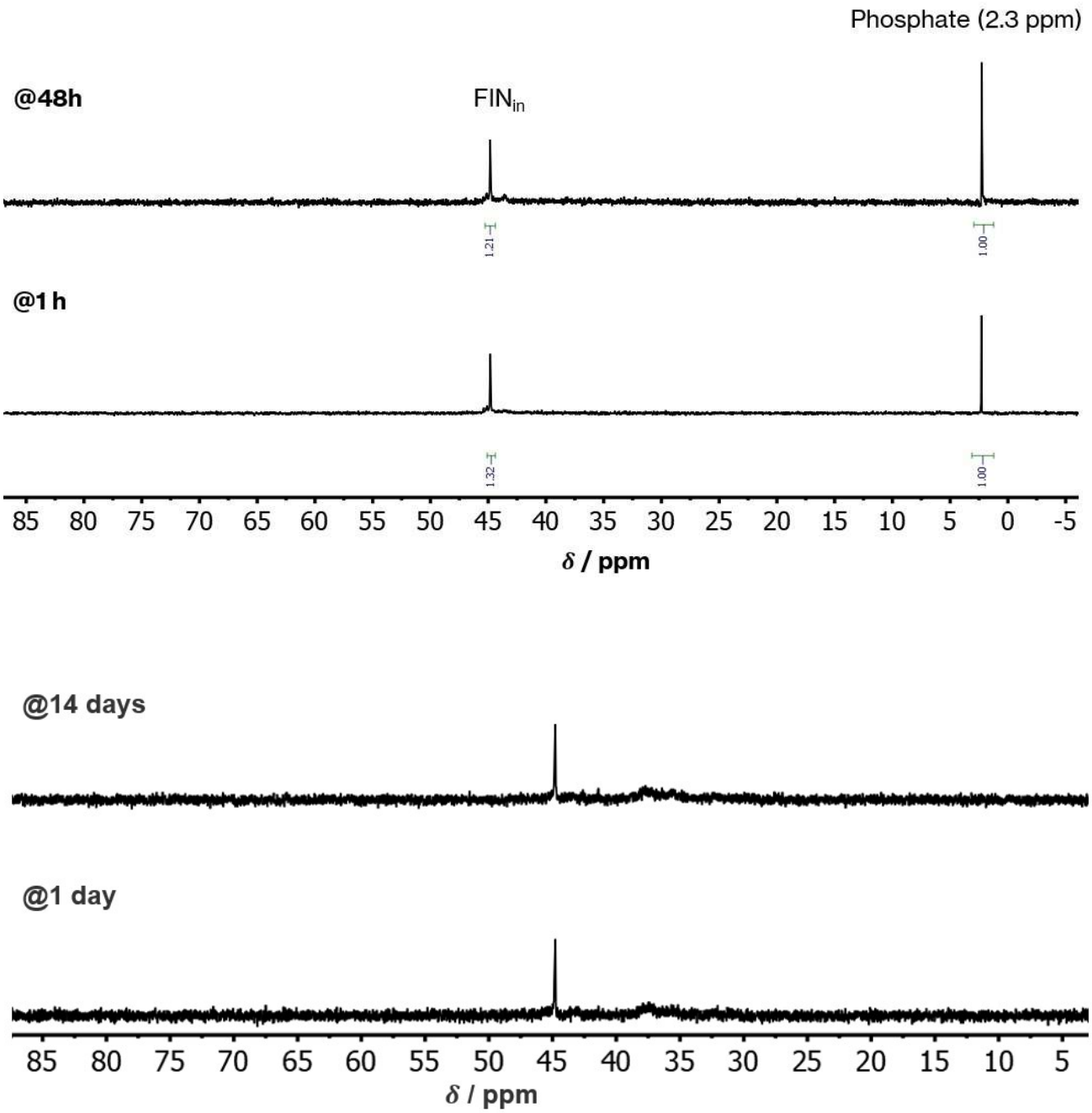


Figure S7. $^{31}P\{^{1}H\}$ NMR (162 MHz) spectra of Ga-FIN (**1a**) at different intervals of time in D_2O (top) with 1X phosphate buffered saline and (bottom) without PBS. Integration shows a negligible change in ^{31}P NMR resonance intensity of cage to standard.

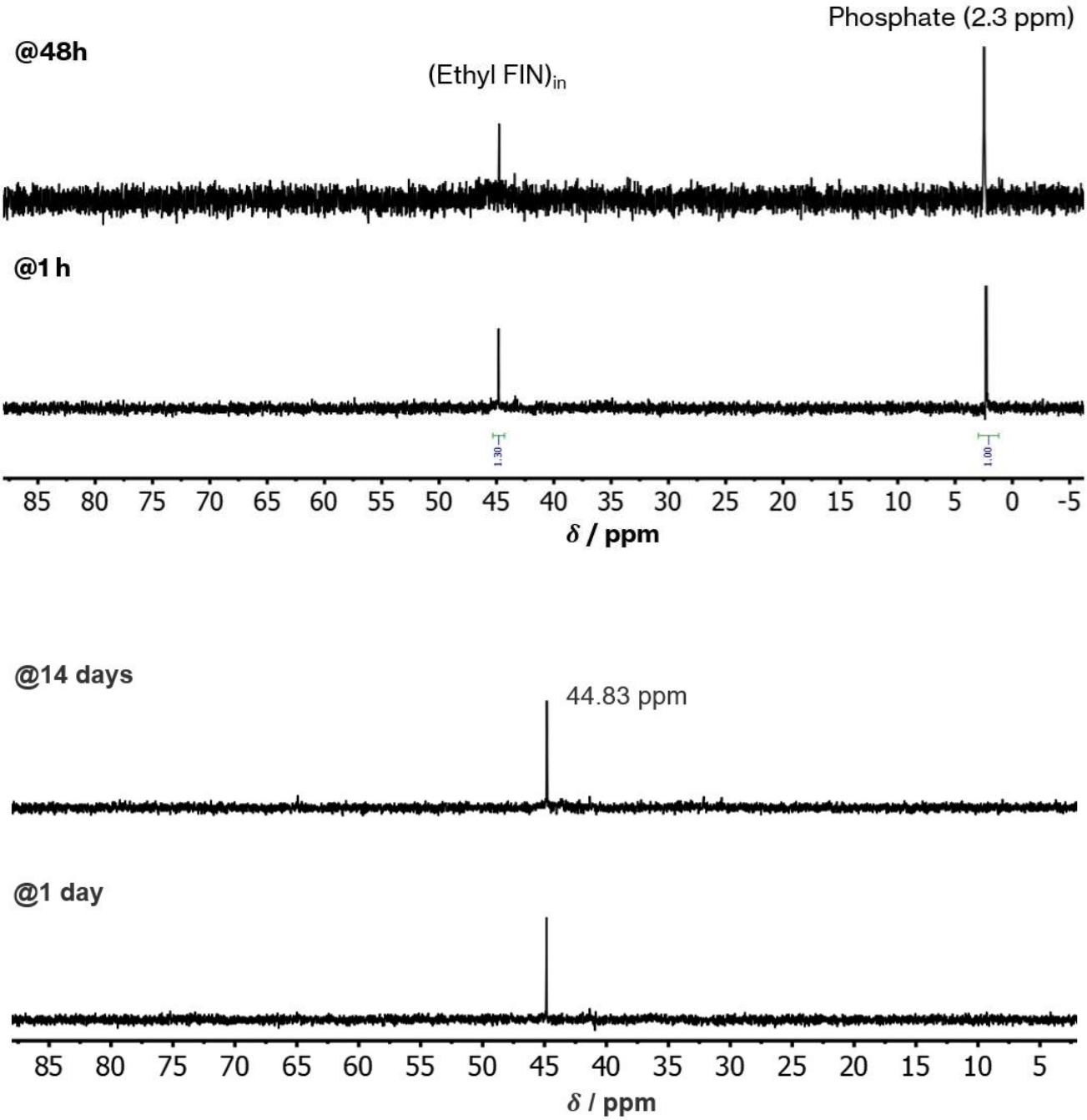


Figure S8. $^{31}P\{^{1}H\}$ NMR (202 MHz) spectra of Ga-EthylFIN (**1b**) at different intervals of time in D_2O (pD = 8.6) (top) with phosphate buffered saline (PBS) and without PBS (bottom).

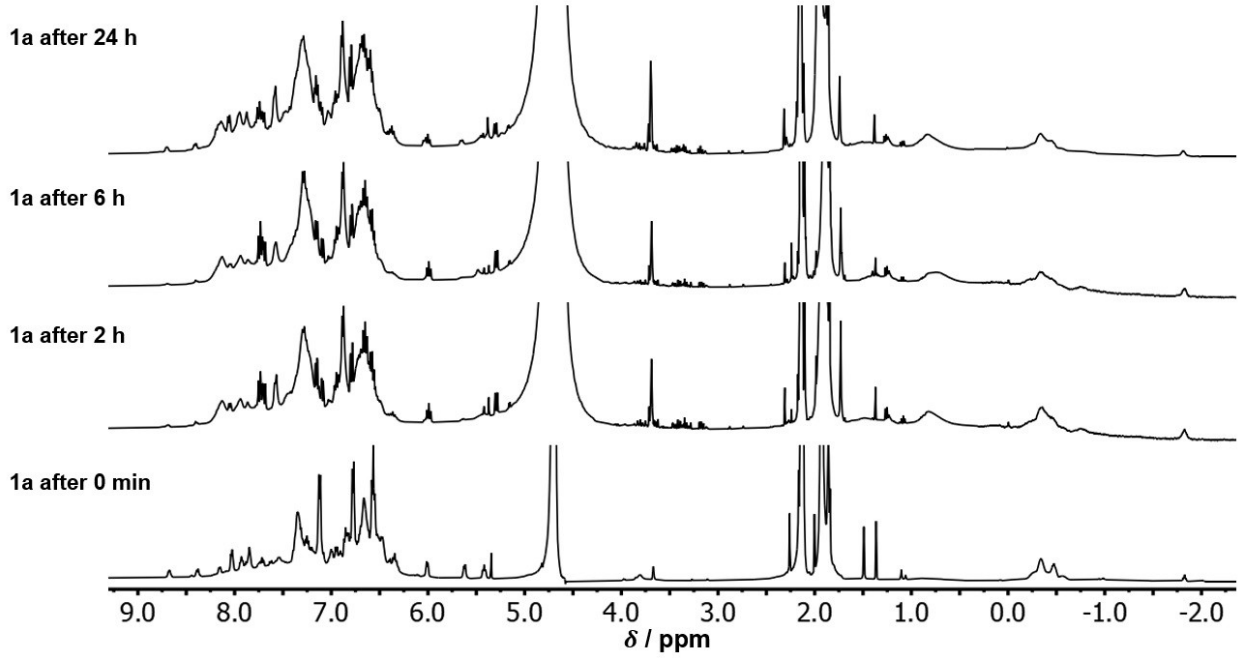


Figure S9. ^{1}H NMR spectra of Ga-FIN (**1a**) at different intervals of time in D_2O in the presence of RPMI 1640 cell media (10% FBS).

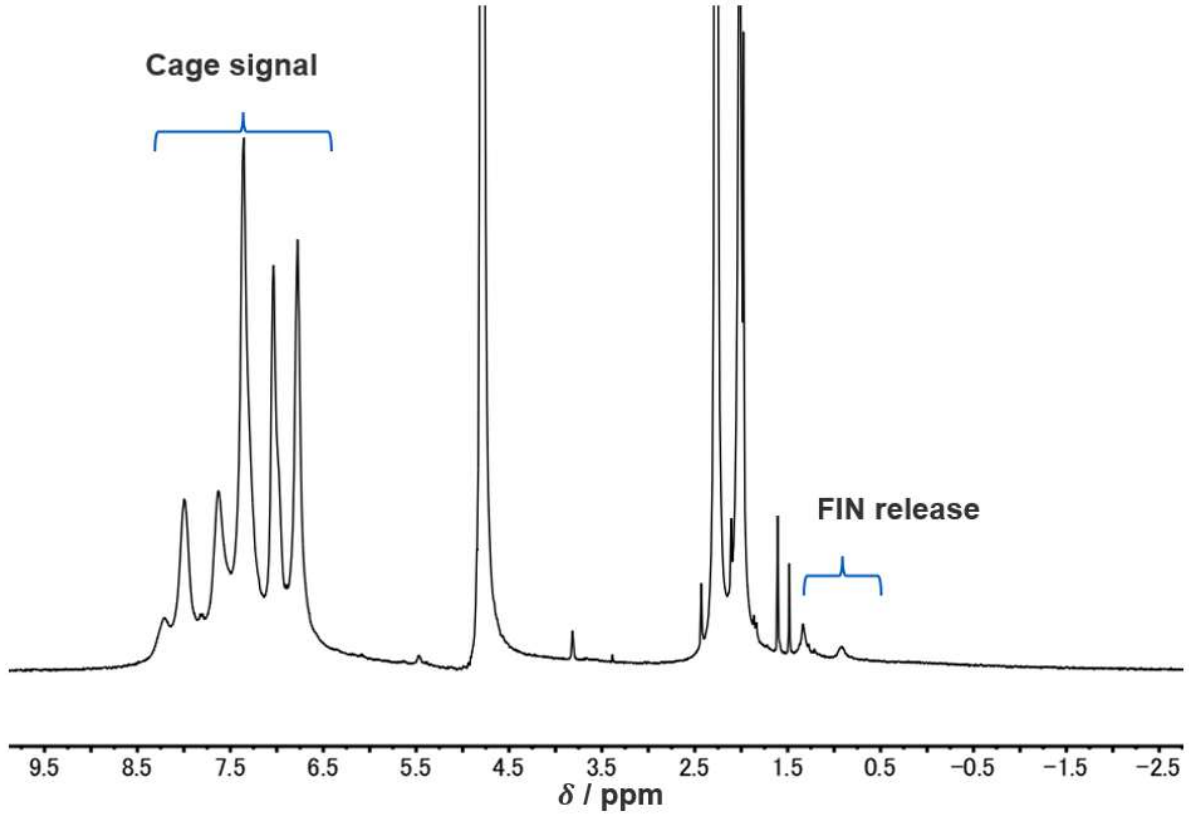


Figure S10. ^{1}H NMR (400 MHz) spectrum of Ga-FIN (**1a**) at pD = 6.2 showing released gold complex in D_2O

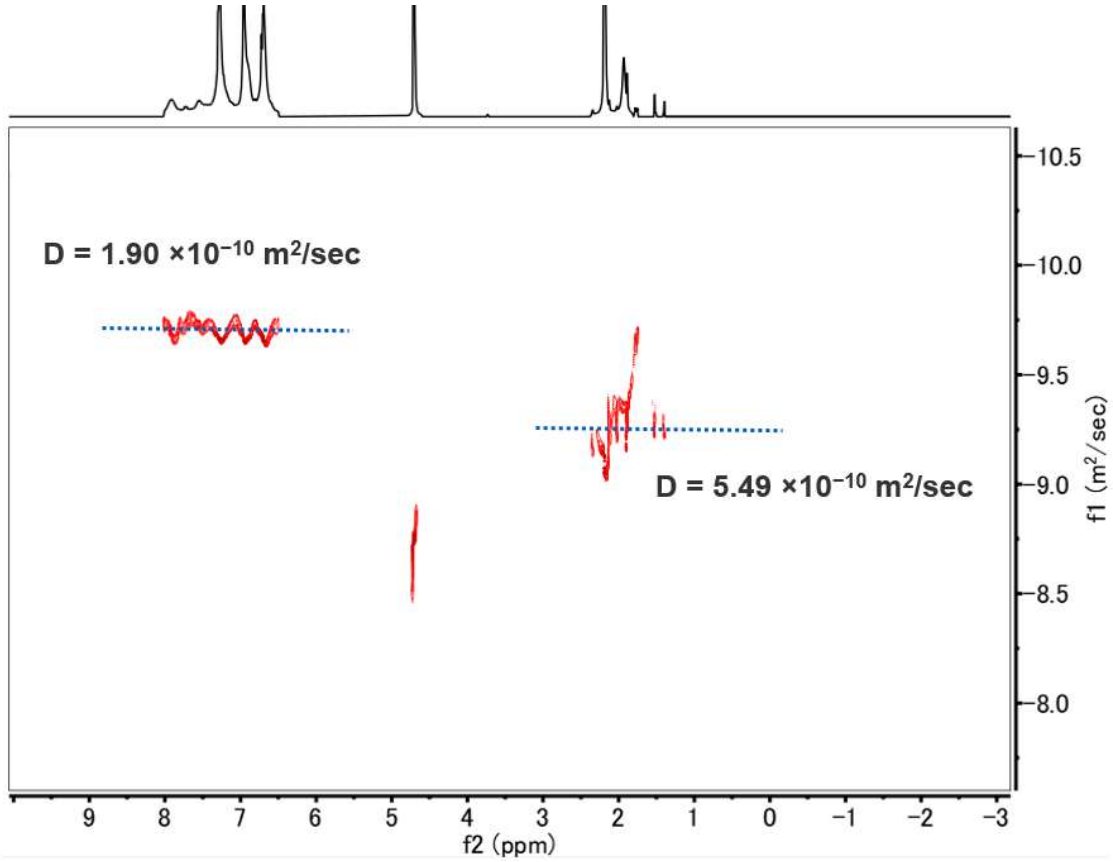


Figure S11. DOSY spectrum (400 MHz) of Ga-FIN (**1a**) in D_2O at pD 6.2 (diffusion constant, D = 1.90 $\times 10^{-10}$ m^2/sec for native cage and D = 5.49 $\times 10^{-10}$ m^2/sec for released gold(I) complex).

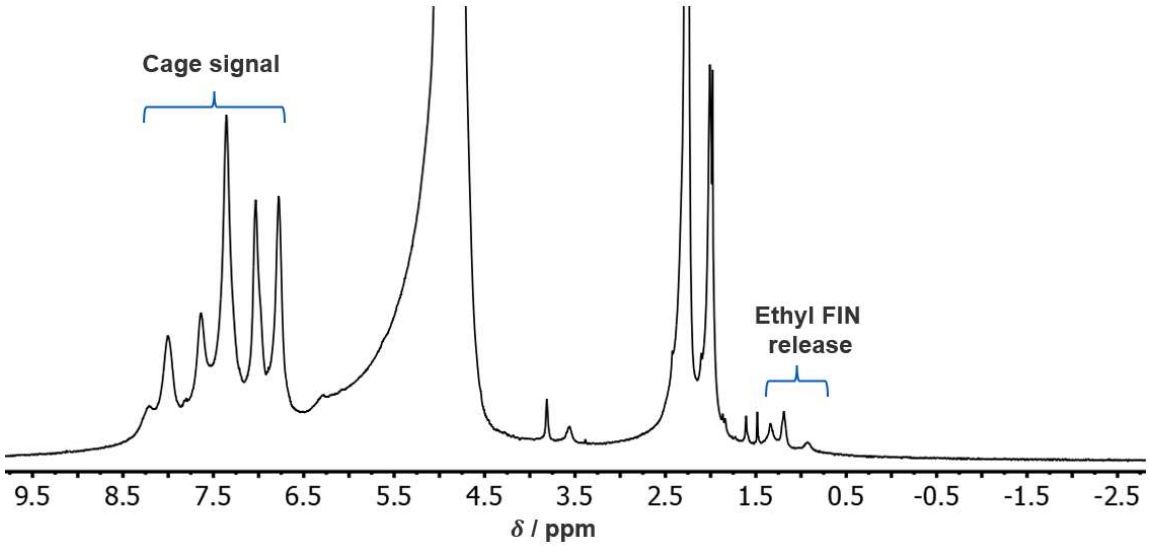


Figure S12. ^1H NMR (400 MHz) spectrum of Ga-EthylFIN (**1b**) at pD = 6.2 showing released gold complex in D_2O.

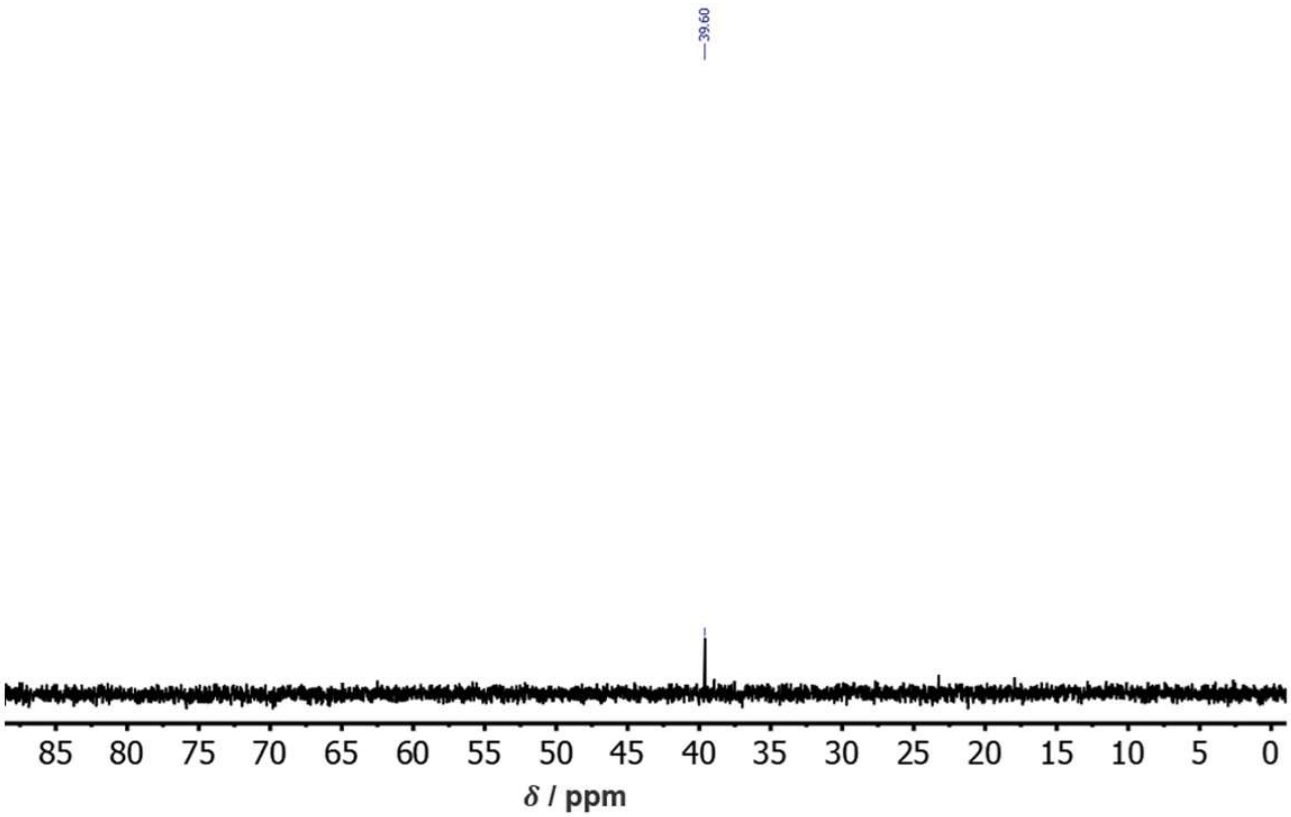


Figure S13. $^{31}P\{^{1}H\}$ NMR (202 MHz) spectrum of Ga-FIN (**1a**) pD 6.2 in D_2O.

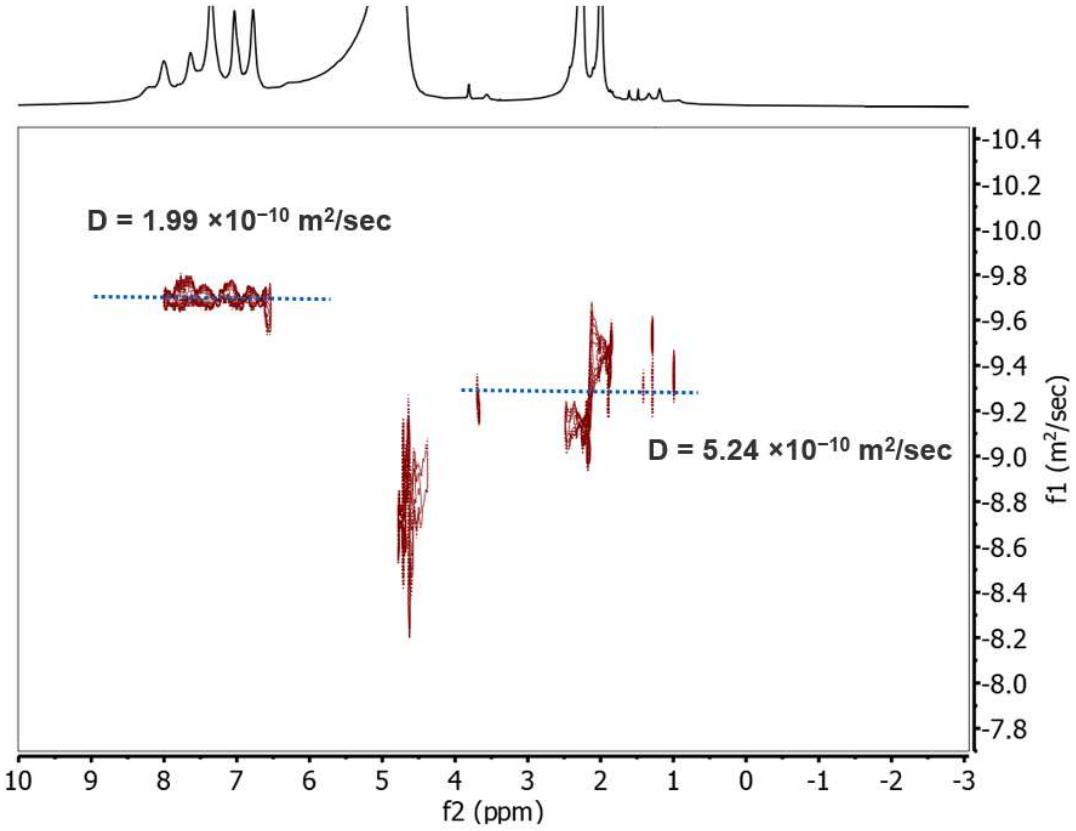


Figure S14. DOSY spectrum (400 MHz) of Ga-EthylFIN (**1b**) in D_2O at pD 6.2 (diffusion constant, $D = 1.99 \times 10^{-10}$ m^2/sec for native cage and $D = 5.24 \times 10^{-10}$ m^2/sec for released gold(I) complex).

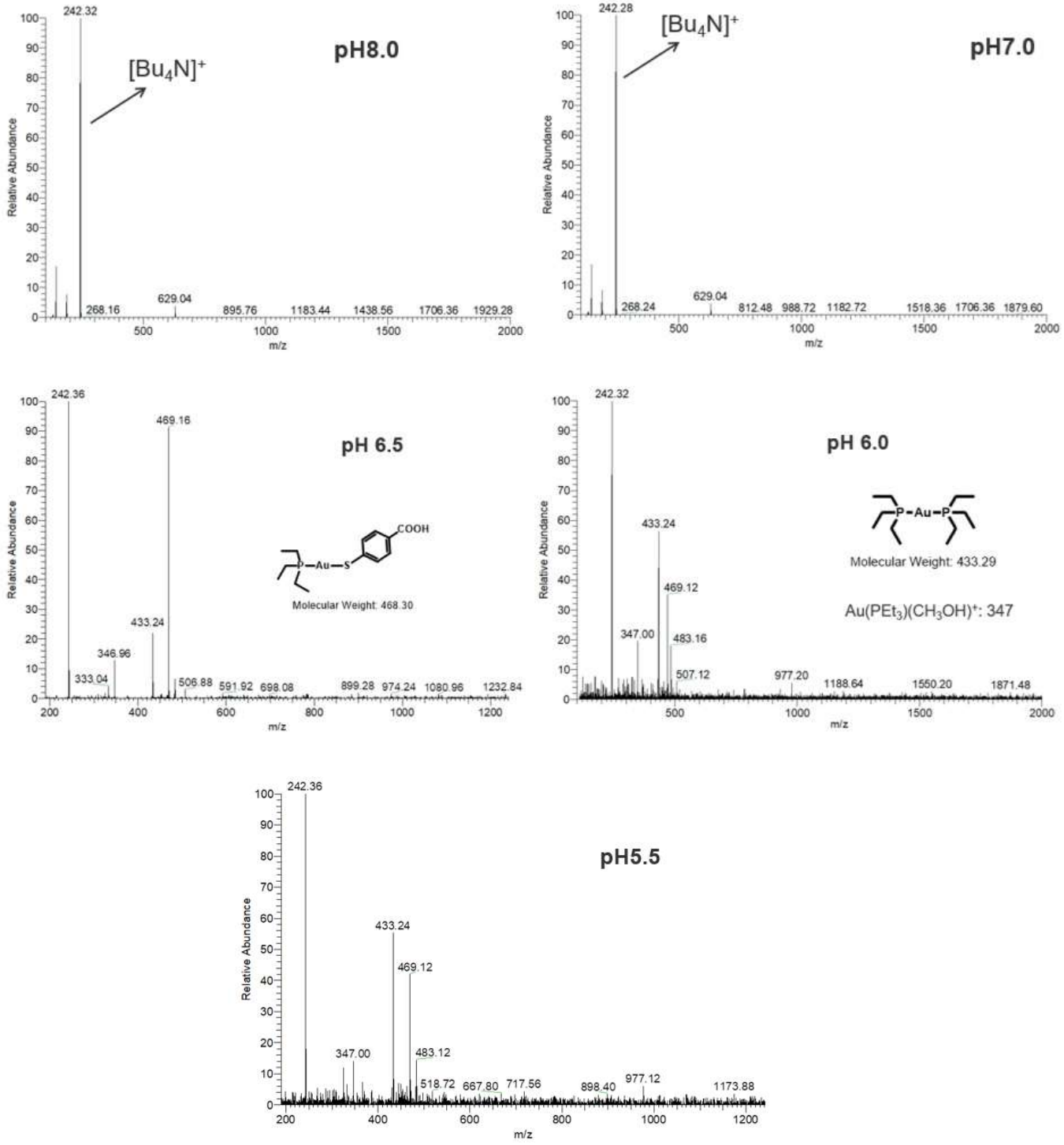


Figure S15. Electrospray (ESI) mass spectra (positive mode) showing release of FIN (gold complex) from a solution containing a 1:1 ratio of Bu_4NPF_6 (as standard) and FIN@1 cage (Fe) at different pH values. Most of the FIN complex was released at pH 6.5. At lower pH values (5.5-6.0), a bis-phosphine gold complex was produced by reaction of encapsulated FIN complex.

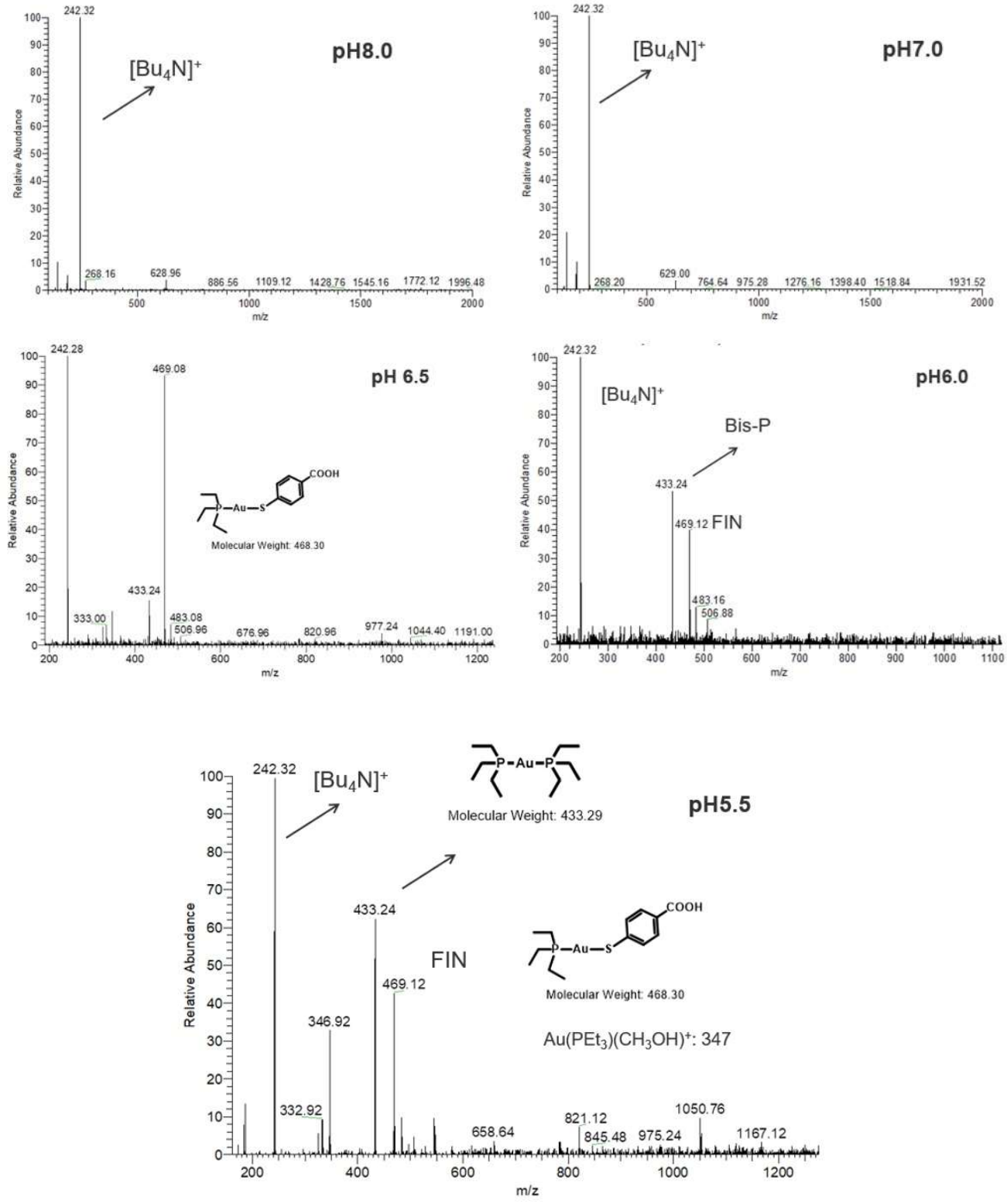


Figure S16. Electrospray (ESI) mass spectra (positive mode) showing release of FIN from a solution containing a 1:1 ratio of Bu_4NPF_6 (as standard) and Ga-FIN (**1a**) at different pH values. Most of the FIN complex was released by pH 6.5. Approximately 40% release of FIN at pH 6.0 and 60% bis-phosphine gold complex under these conditions.

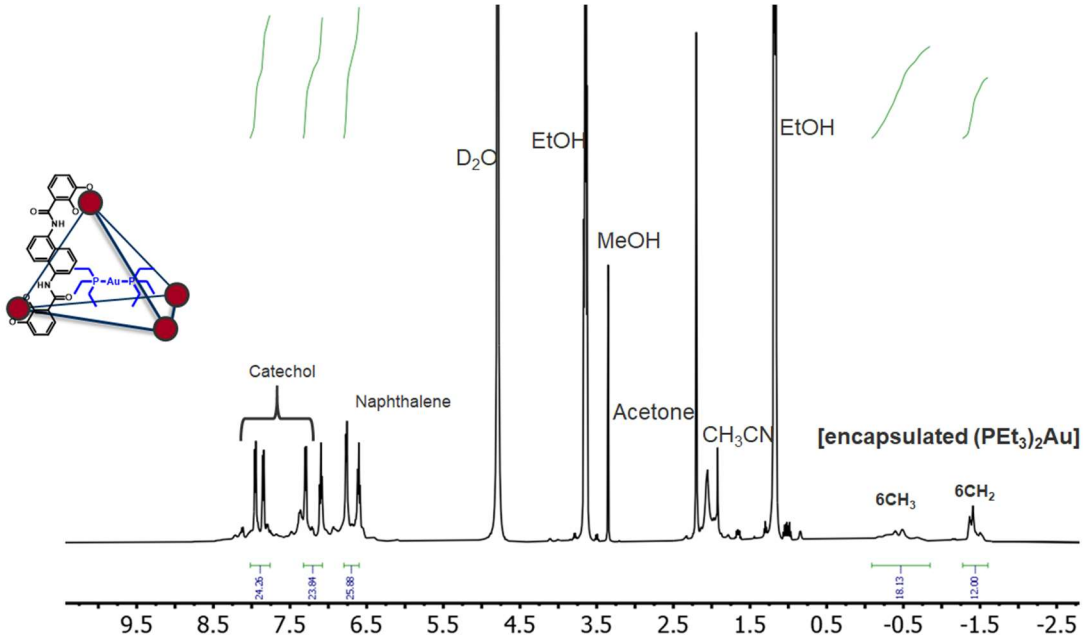


Figure S17. ^{1}H NMR (500 MHz) of Ga-Au(PEt_3)$_2$ (**1c**) (in D_2O.

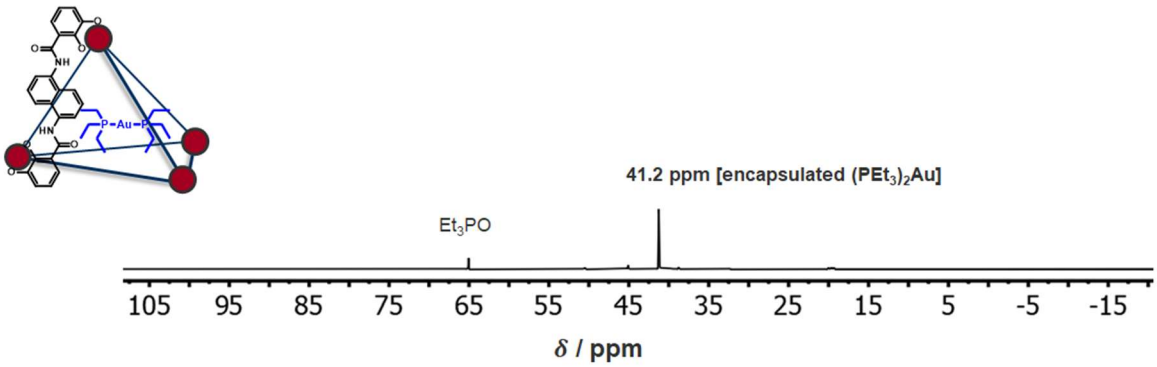


Figure S18. ^{31}P{1H} NMR (202 MHz) spectrum of Ga-Au(PEt_3)$_2$ (**1c**) in D_2O.

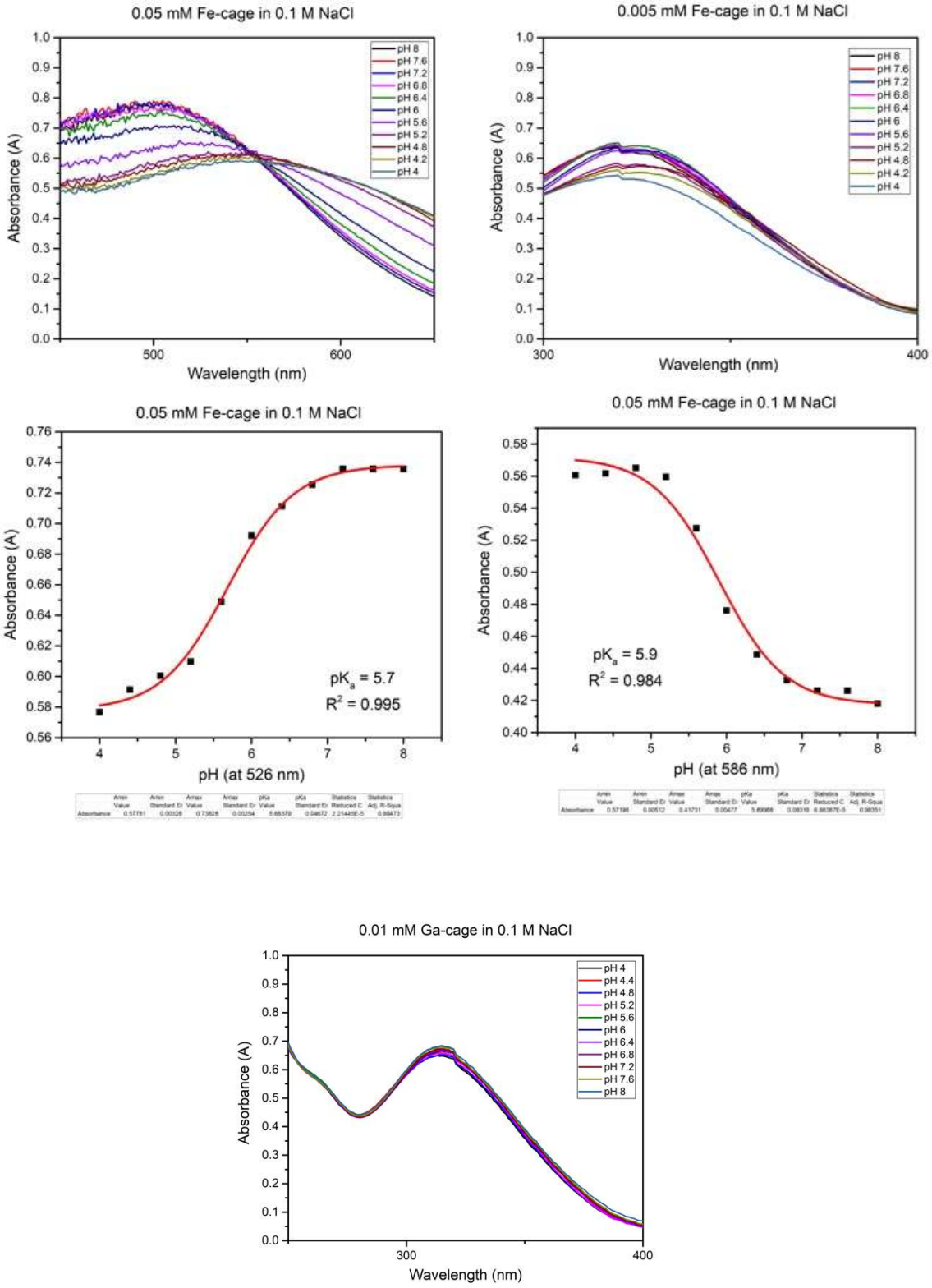
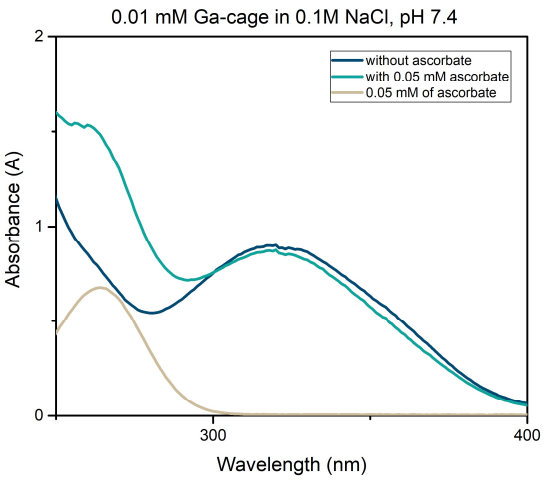
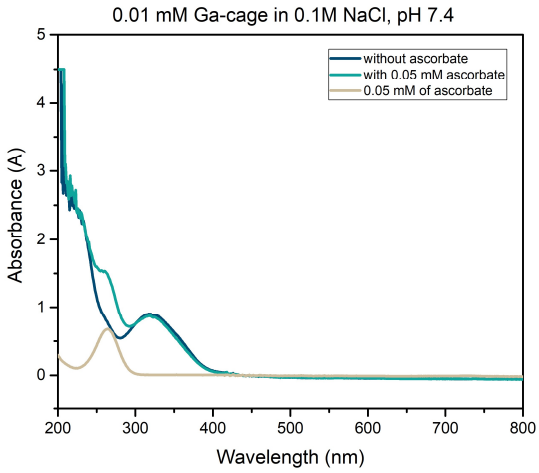
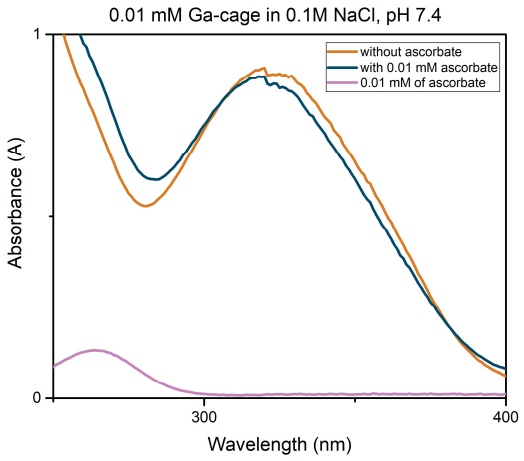
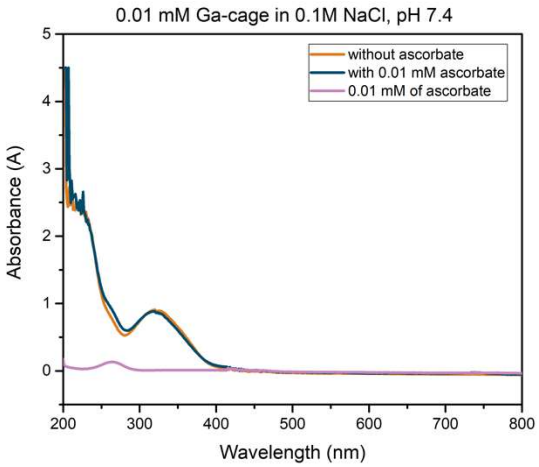


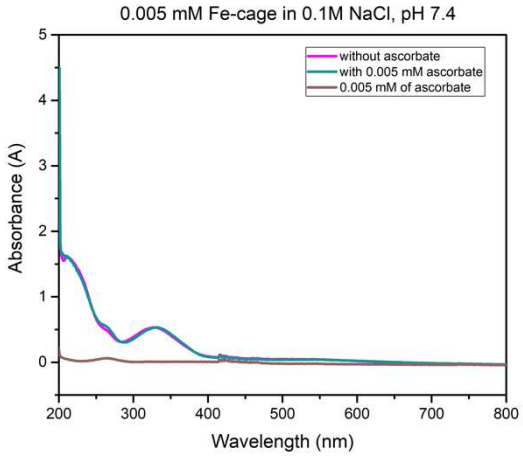
Figure S19. Top: electronic absorption spectrum as a function of pH and plot of data to obtain pK_a of Fe(III) cage (**2**) in 0.10 M NaCl. Bottom: Electronic absorption spectrum as a function of pH for Ga(III) cage (**1**) in 0.1 M NaCl, 37 °C.

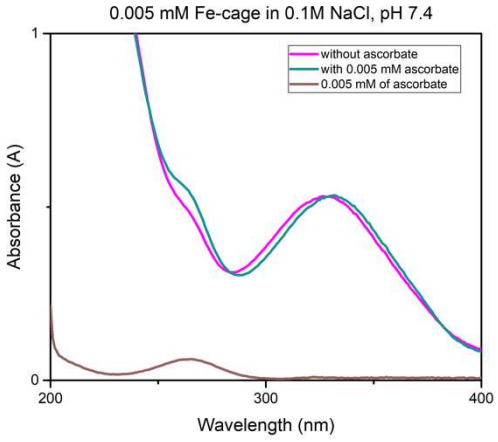

0.01 mM Ga-cage in 0.1M NaCl, pH 7.4
without ascorbate
with 0.05 mM ascorbate
0.05 mM of ascorbate
Absorbance (A)
Wavelength (nm)


0.01 mM Ga-cage in 0.1M NaCl, pH 7.4
without ascorbate
with 0.05 mM ascorbate
0.05 mM of ascorbate
Absorbance (A)
Wavelength (nm)


0.01 mM Ga-cage in 0.1M NaCl, pH 7.4
without ascorbate
with 0.01 mM ascorbate
0.01 mM of ascorbate
Absorbance (A)
Wavelength (nm)


0.01 mM Ga-cage in 0.1M NaCl, pH 7.4
without ascorbate
with 0.01 mM ascorbate
0.01 mM of ascorbate
Absorbance (A)
Wavelength (nm)


0.005 mM Fe-cage in 0.1M NaCl, pH 7.4
without ascorbate
with 0.005 mM ascorbate
0.005 mM of ascorbate
Absorbance (A)
Wavelength (nm)


0.005 mM Fe-cage in 0.1M NaCl, pH 7.4
without ascorbate
with 0.005 mM ascorbate
0.005 mM of ascorbate
Absorbance (A)
Wavelength (nm)

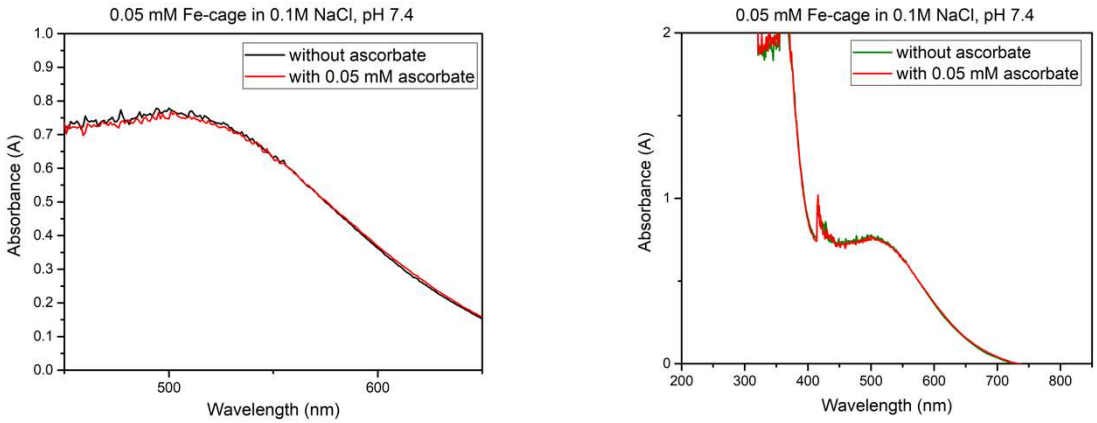


Figure S20 Electronic absorption spectra of (top) Ga(III) cage and (bottom) Fe(III) cage in the presence of ascorbate in 0.1 M NaCl.

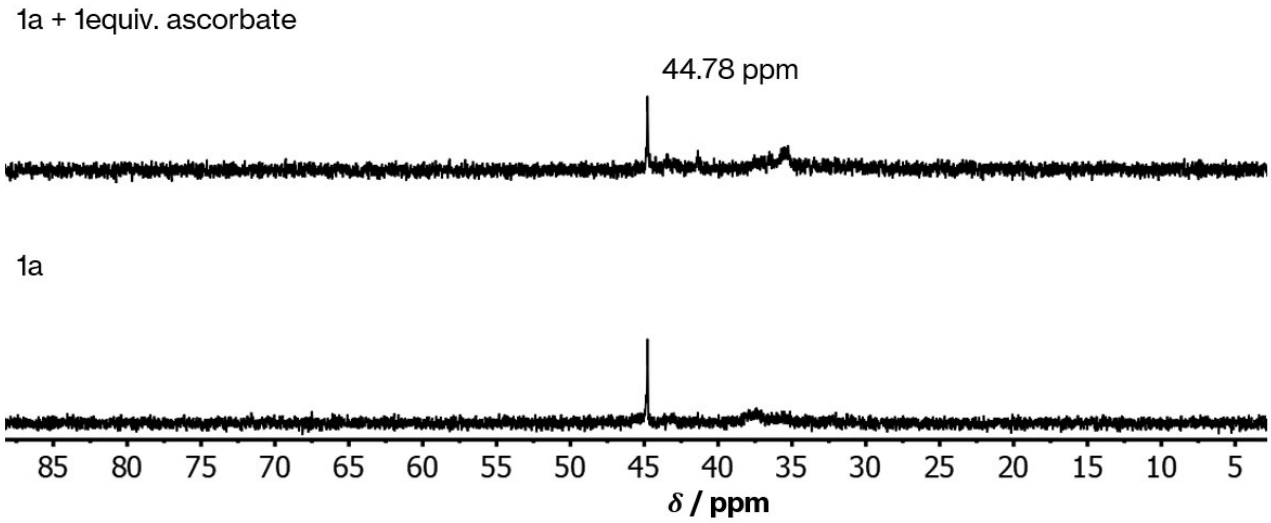


Figure S21. ^{31}P NMR spectroscopy studies on the stability of Ga-FIN (**1a)** in the presence of ascorbic acid in D_2O.

Cell viability experiments at different pH values

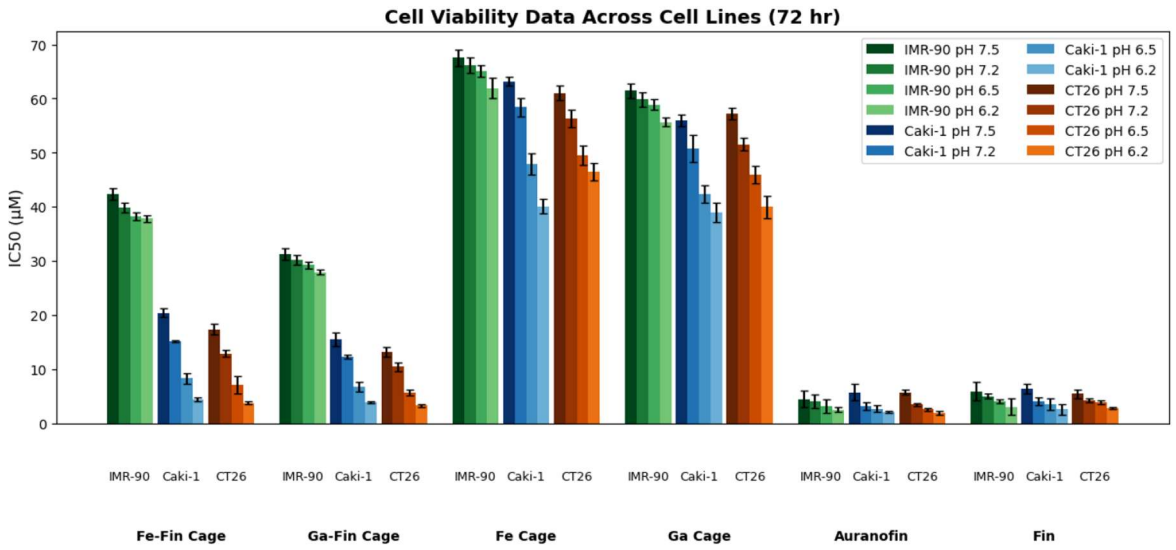


Figure S22 Bar diagram showing the IC_{50} values of gold(I) encapsulated cages, native cages and control complexes such as Auranofin and FIN against IMR-90, Caki-1 and CT26 at four different pH values after 72h incubation.

Table S1. Mean IC_{50} values $\pm$ SD (μM) on Caki-1 cells after 24h incubation from three independent experiments. Cages (**1** and **2**) and metal-containing cages (**1a** and **2a**) were dissolved in H_2O and Auranofin and Fin in DMSO (0.1%). Dilutions were performed in media.

pH Level	Ga-Cage (1)	Fe-Cage (2)	Ga-FIN Cage (1a)	Fe-FIN Cage (2a)	Auranofin	Fin
7.5	65.97 ± 1.27	79.38 ± 1.59	23.92 ± 1.03	31.37 ± 1.19	7.35 ± 0.59	8.82 ± 0.87
7.2	59.76 ± 1.35	70.15 ± 1.87	18.87 ± 0.92	23.34 ± 0.71	5.33 ± 0.33	6.60 ± 0.44
6.5	53.32 ± 1.87	65.61 ± 2.17	10.33± 0.67	12.78 ± 1.88	4.71 ± 0.32	5.94 ± 0.38
6.2	50.02 ± 2.41	58.11 ± 1.90	5.93 ± 0.28	6.81± 0.33	3.46 ± 0.51	4.12 ± 0.17

Table S2. Mean IC_{50} values ± SD (μM) on Caki-1 cells after 72h incubation from three independent experiments. Cages (**1** and **2**) and metal-containing cages (**1a** and **2a**) were dissolved in H_2O and Auranofin and Fin in DMSO (0.1%). Dilutions were performed in media.

pH Level	Ga-Cage (1)	Fe-Cage (2)	Ga-FIN Cage (1a)	Fe-FIN Cage (2a)	Auranofin	Fin
7.5	55.97 ± 1.07	63.20 ± 0.82	15.55 ± 1.22	20.39 ± 0.77	5. 73 ± 1.52	6.38 ± 0.91
7.2	50.76 ± 2.51	58.40 ± 1.64	12.27 ± 0.41	15.17 ± 0.23	3.11 ± 0.74	4.03 ± 0.75
6.5	42.32 ± 1.62	47.91 ± 1.97	6.72 ± 0.87	8.31 ± 1.01	2.71 ± 0.54	3.51 ± 1.02
6.2	39.02 ± 1.81	40.11 ± 1.38	3.86 ± 0.11	4.43 ± 0.35	2.06 ± 0.21	2.57 ± 0.97

Table S3. Mean IC_{50} values ± SD (μM) on CT26 cells after 72h incubation from three independent experiments. Cages (**1** and **2**) and metal-containing cages (**1a** and **2a**) were dissolved in H_2O and Auranofin and Fin in DMSO (0.1%). Dilutions were performed in media.

pH Level	Ga-Cage (1)	Fe-Cage (2)	Ga-FIN Cage (1a)	Fe-FIN Cage (2a)	Auranofin	Fin
7.5	57.29 ± 1.08	61.02 ± 1.35	13.22 ± 0.88	17.33 ± 1.01	5.72 ± 0.50	5.42 ± 0.74
7.2	51.55 ± 1.15	56.29 ± 1.59	10.43 ± 0.78	12.89 ± 0.60	3.49 ± 0.28	4.20 ± 0.37
6.5	45.99 ± 1.59	49.56 ± 1.84	5.71 ± 0.57	7.06 ± 1.60	2.60 ± 0.27	3.83 ± 0.32
6.2	39.95 ± 2.05	46.42 ± 1.62	3.28 ± 0.24	3.77 ± 0.28	1.91 ± 0.43	2.83 ± 0.14

Table S4. Mean IC_{50} values ± SD (μM) on IMR-90 cells after 24h incubation from three independent experiments. Cages (**1** and **2**) and metal-containing cages (**1a** and **2a**) were dissolved in H_2O and Auranofin and Fin in DMSO (0.1%). Dilutions were performed in media.

pH Level	Ga-Cage (1)	Fe-Cage (2)	Ga-FIN Cage (1a)	Fe-FIN Cage (2a)	Auranofin	Fin
7.5	81.91 ± 1.54	89.80 ± 1.77	47.91 ± 1.12	65.74 ± 1.32	8.52 ± 1.59	9.12 ± 1.87
7.2	79.96 ± 1.42	87.27 ± 1.64	45.43 ± 0.93	63.08 ± 1.08	6.81 ± 1.33	7.97 ± 1.64
6.5	77.35 ± 1.01	85.82 ± 1.23	42.89 ± 0.72	61.61 ± 0.89	5.65 ± 0.32	6.83 ± 1.85
6.2	75.31 ± 0.88	84.43 ± 1.09	41.15 ± 2.49	60.94 ± 1.65	4.87 ± 0.51	5.79 ± 0.96

Table S5. Mean IC_{50} values ± SD (μM) on IMR-90 cells after 72h incubation from three independent experiments. Cages (**1** and **2**) and metal-containing cages (**1a** and **2a**) were dissolved in H_2O and Auranofin and Fin in DMSO (0.1%). Dilutions were performed in media.

pH Level	Ga-Cage (1)	Fe-Cage (2)	Ga-FIN Cage (1a)	Fe-FIN Cage (2a)	Auranofin	Fin
7.5	61.44 ± 1.41	67.53 ± 1.52	31.25 ± 1.05	42.36 ± 1.10	4.45 ± 1.53	5.93 ± 1.71
7.2	59.82 ± 1.29	66.21 ± 1.38	30.12 ± 0.89	39.89 ± 0.95	4.03 ± 1.29	4.98 ± 0.42
6.5	58.91 ± 0.98	65.06 ± 1.07	29.23 ± 0.58	38.24 ± 0.76	3.13 ± 1.31	4.01 ± 0.37
6.2	55.67 ± 0.77	61.94 ± 1.89	27.92 ± 0.45	37.88 ± 0.62	2.49 ± 0.44	3.02 ± 1.526

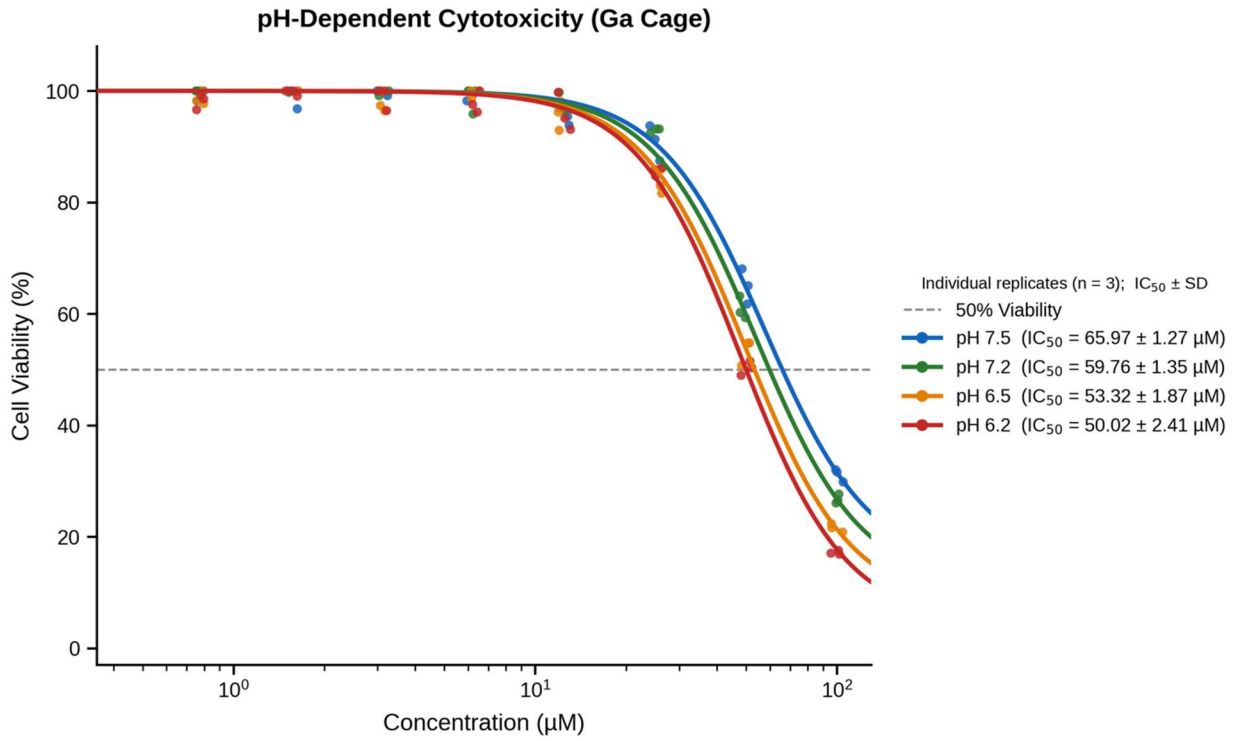


Figure S23. pH-dependent cell viability profile of **Ga cage (1)** at different concentrations on human clear cell renal cell carcinoma (Caki-1) cells (Presto-Blue assay). Individual data points from three independent experiments (n = 3) are shown. IC_{50} values ± SD are reported in the legend; the dashed line indicates 50% viability.

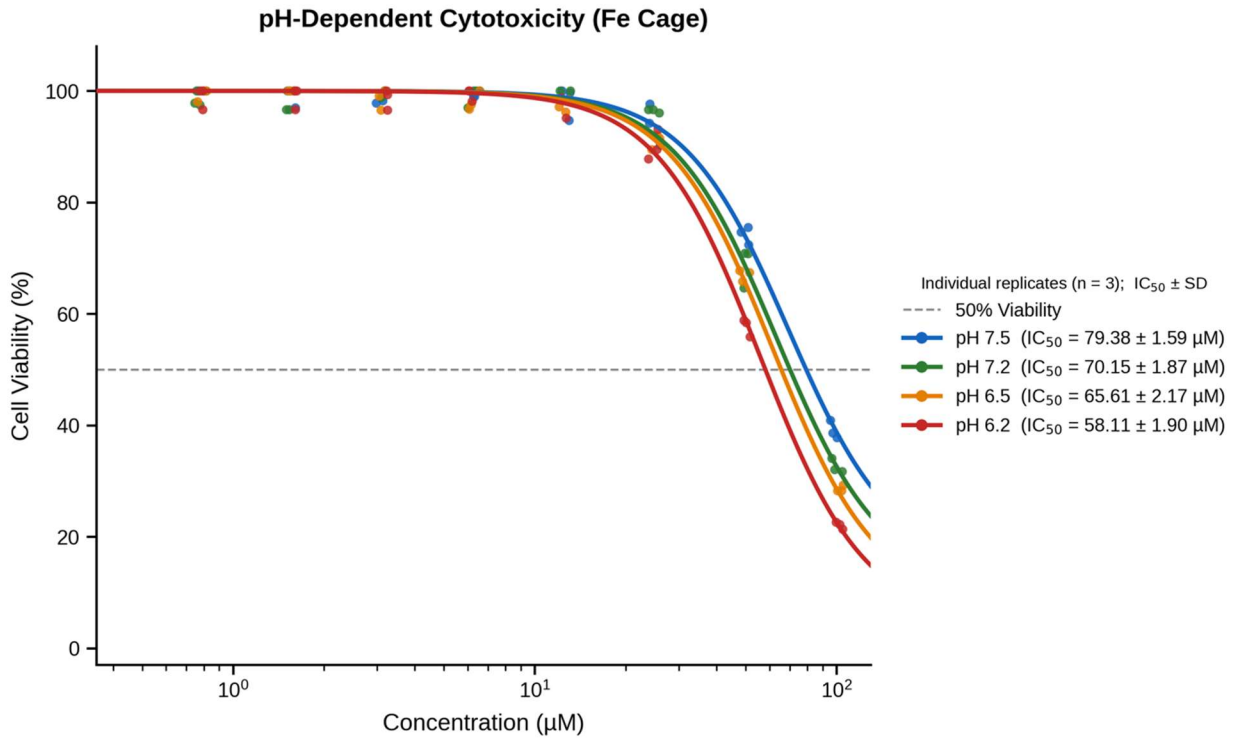


Figure S24. pH-dependent cell viability profile of **Fe cage (2)** at different concentrations on human clear cell renal cell carcinoma (Caki-1) cells (Presto-Blue assay). Individual data points from three independent experiments (n = 3) are shown. IC_{50} values ± SD are reported in the legend; the dashed line indicates 50% viability.

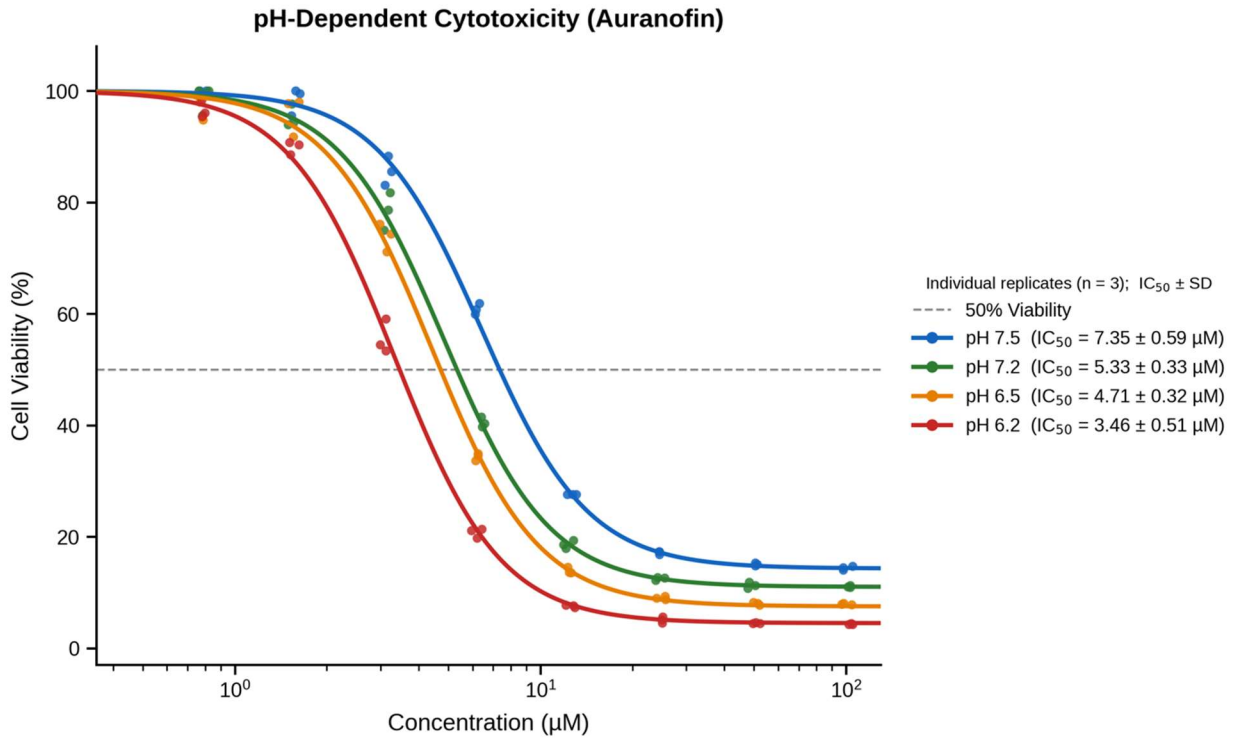


Figure S25. pH-dependent cell viability profile **Auranofin** at different concentrations on human clear cell renal cell carcinoma (Caki-1) cells (Presto-Blue assay). Individual data points from three independent experiments (n = 3) are shown. IC_{50} values ± SD are reported in the legend; the dashed line indicates 50% viability.

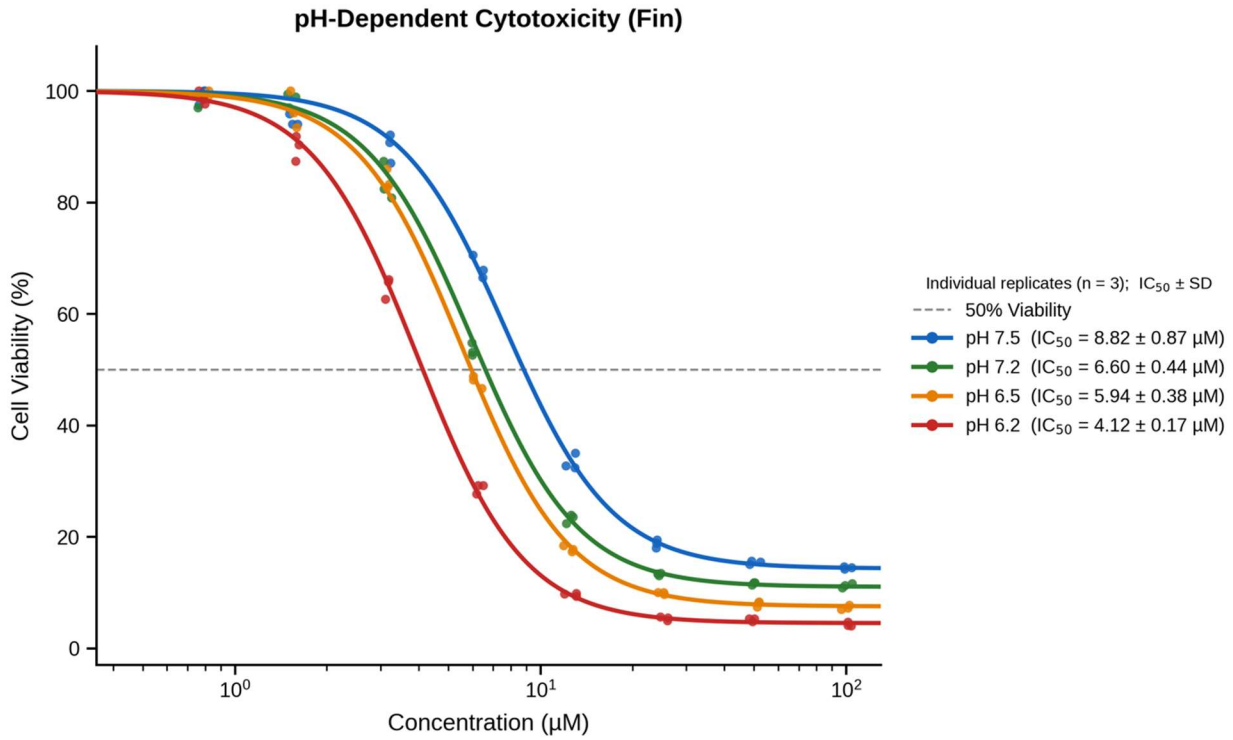


Figure S26. pH-dependent cell viability profile **FIN** at different concentrations on human clear cell renal cell carcinoma (Caki-1) cells (Presto-Blue assay). Individual data points from three independent experiments (n = 3) are shown. IC_{50} values ± SD are reported in the legend; the dashed line indicates 50% viability.

Gold Content Analysis in Whole Cell and Organelle CT26 Lysates by ICP-MS)

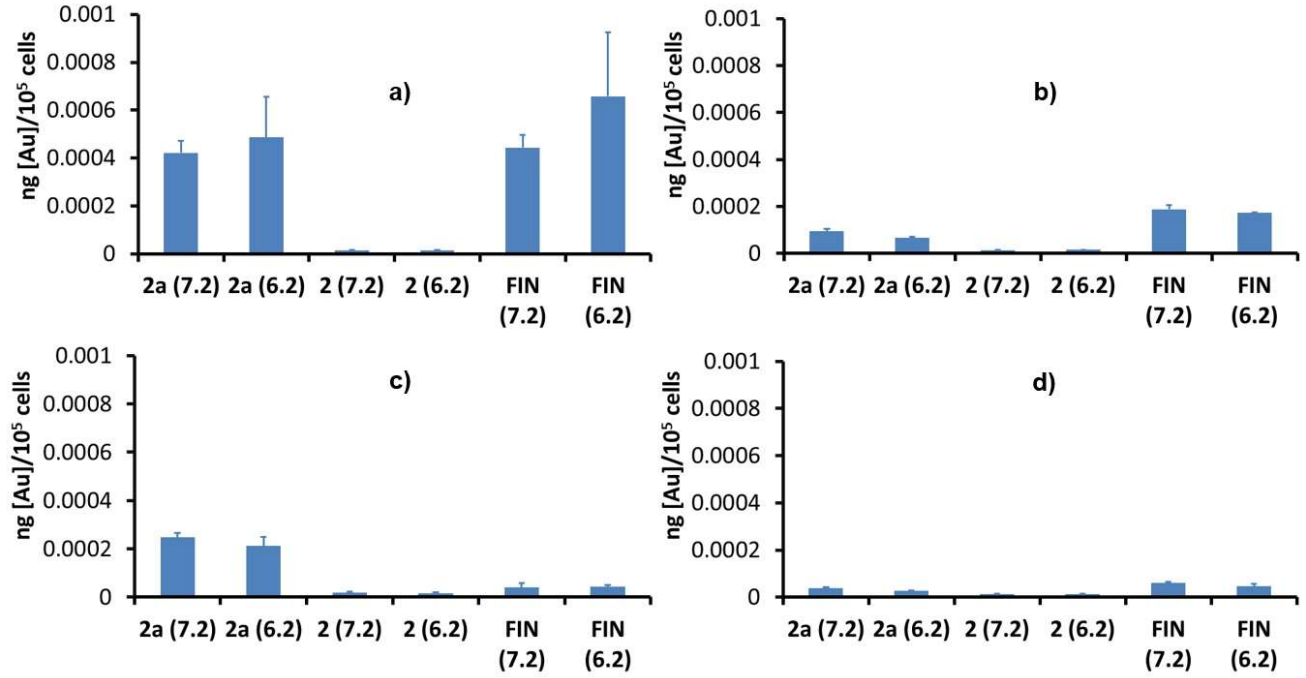


Figure S27. Accumulation of Au in (a) whole cell (b) cytoplasm, (c) nucleus and (d) mitochondria (CT26 cell line) as measured by ICP-MS upon treatment with Fe(III) cage with encapsulated FIN (**2a**) and Fe(III) cage (**2**). The respective pH values are shown in parentheses.

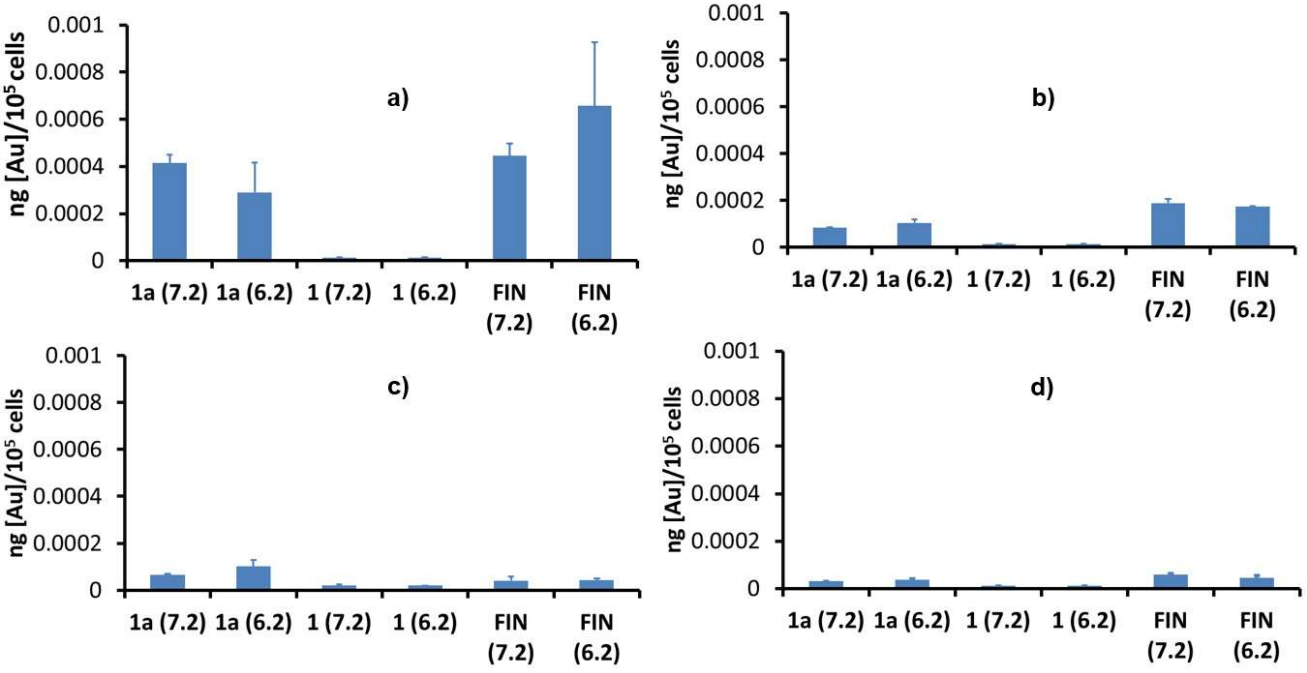


Figure S28. Accumulation of Au in (a) whole cell (b) cytoplasm, (c) nucleus and (d) mitochondria (CT26 cell line) as measured by ICP-MS upon treatment with Ga(III) cage with encapsulated FIN (**1a**) and Ga(III) cage (**1**). The respective pH values are shown in parentheses.

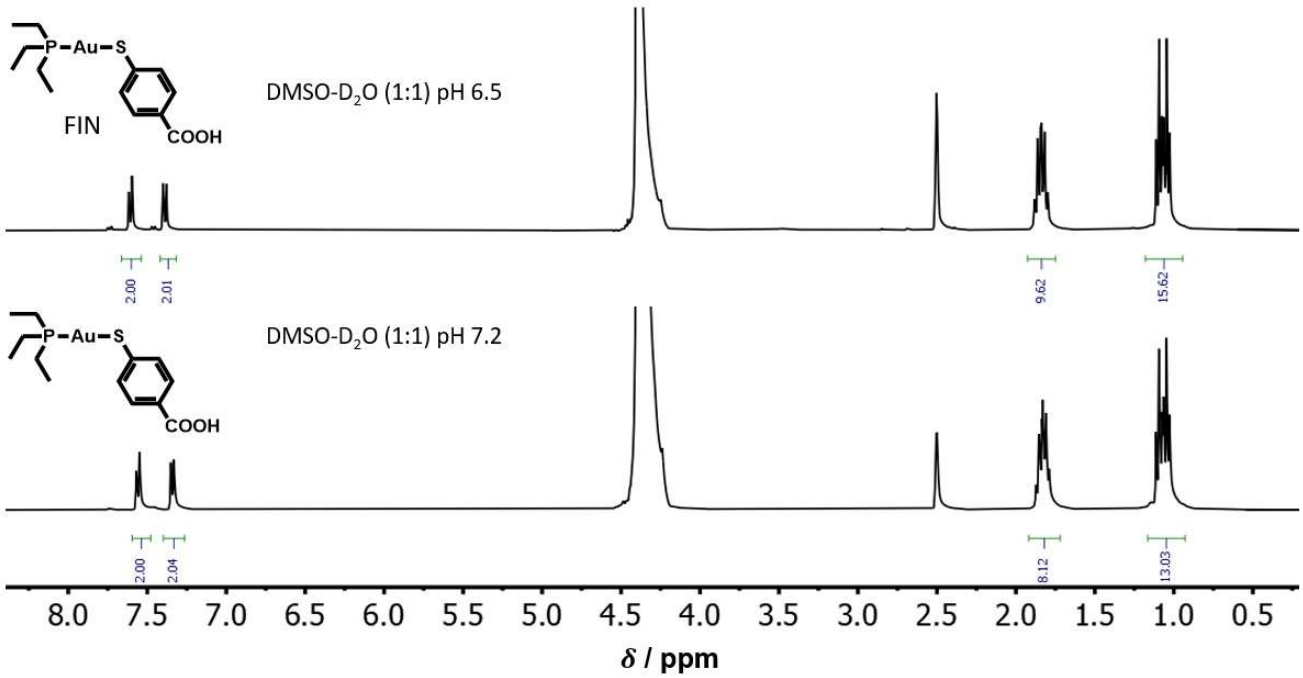


Figure S29. ^{1}H NMR (400 MHz) of FIN complex with pD ≈ 7.2 and 6.5 in DMSO-D_6:D_2O (1:1).

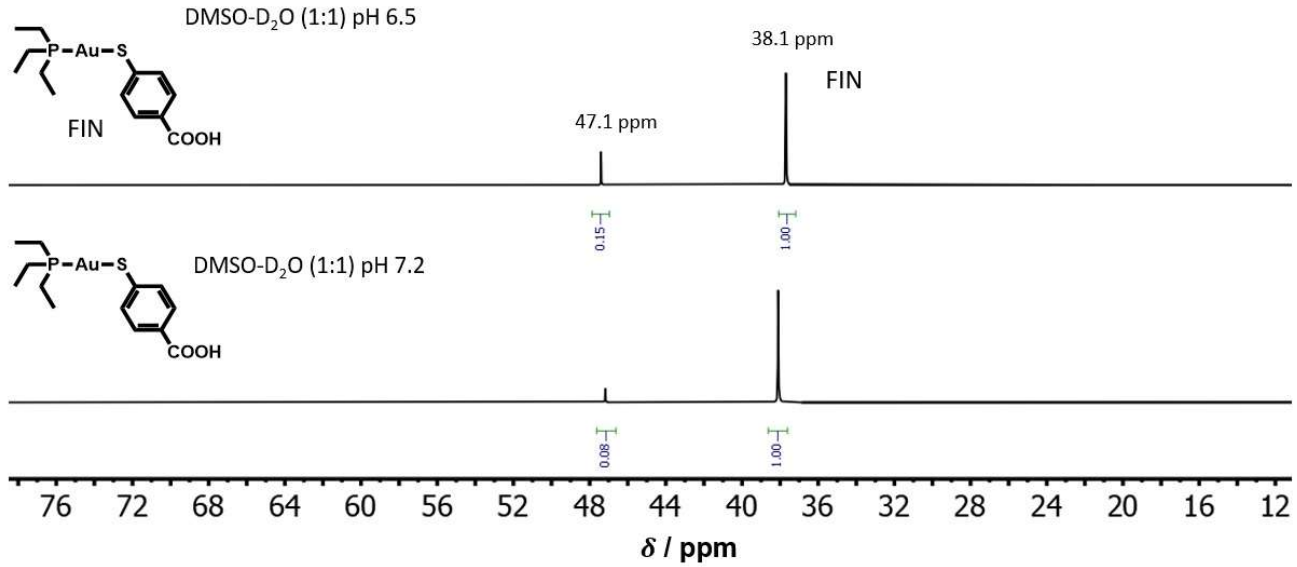


Figure S30. $^{31}P\{^{1}H\}$ NMR of FIN complex at pD ≈7.2 and 6.5 in DMSO-D_6:D_2O (1:1).

References

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