Supporting Information

Fluorescence Lifetime Imaging of Upper Gastrointestinal pH *In Vivo* with a Lanthanide Based Near-Infrared τ Probe

Yingying Ning,[a] Shengming Cheng,[b] Jingxiang Wang,[a] Yi-Wei Liu,[a] Wei Feng,[b] Fuyou Li,*[,b] and Jun-Long Zhang*[,a]

[a]Beijing National Laboratory for Molecular Sciences, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, P. R. China.

[b]Department of Chemistry & State Key Laboratory of Molecular Engineering of Polymers & Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, P. R. China

E-mail: zhangjunlong@pku.edu.cn; fyli@fudan.edu.cn

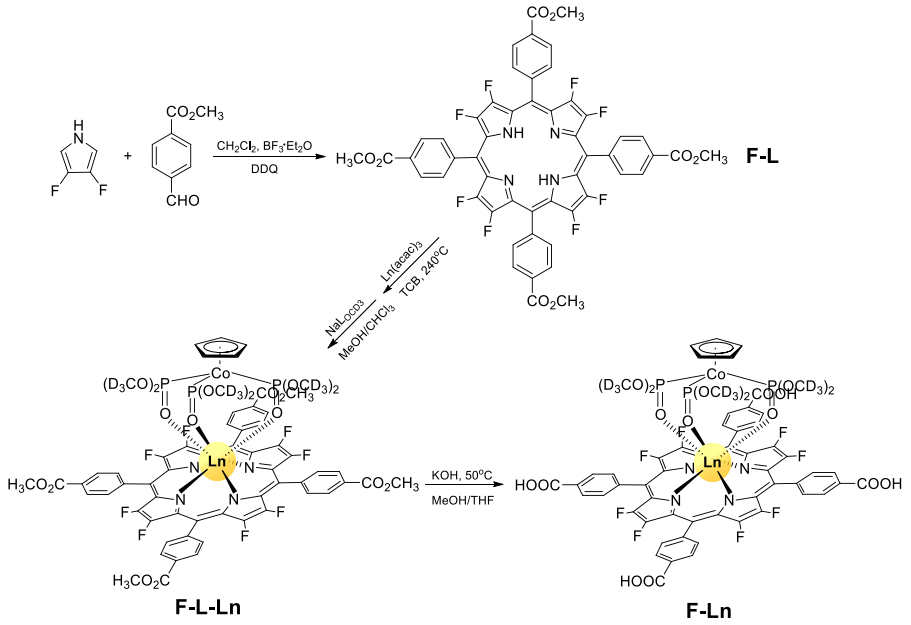
General materials and methods

Unless otherwise stated, all reactions were performed under an inert atmosphere of nitrogen. UV-vis spectra were recorded on an Agilent 8453 UV-vis Spectrometer equipped with an Agilent 89090A Thermostat (±0.1 ^{o}C) at 25 ^{o}C. IR spectra were recorded on Bruker Tensor 27 FT-IR or PE Spectrum Spotlight 200 FT-IR Microscopy. Mass spectra were recorded on Bruker APEX IV FT-ICR Mass Spectrometer (ESI) or Solarix XR Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Simulated mass spectra were obtained at the website http://www.chemcalc.org. NMR spectra were recorded on Bruker ARX400 400MHz or AVANCE III 500MHz NMR Spectrophotometer. Transmission electron microscopy was obtained in JEOL JEM-2100F Field-emission High Resolution Transmission Electron Microscope. Dynamic light scattering was carried out in ALV/DLS/SLS-5022F Laser Light Scattering Spectrometer. Transient absorption spectra and triplet excited state decay dynamics were recorded on an Edinburgh LP920 spectrometer combined with OPO laser excitation pulse (10 Hz, 2 mJ pulse^{-1}). Phosphorescence spectra were recorded on frozen glasses of solutions of Gd^{3+} complexes (MeOH/EtOH 1:1, v/v) using a dewar cuvette filled with liquid N_2 (T = 77 K). HeLa cells were obtained from Peking University Health Science Center. All animal procedures were approved by the Institutional Animal Care and Use Committee of Fudan University. For the optical measurements in liquid solution, spectroscopic-grade dimethyl sulfoxide was used as purchased from Alfa-Aesar. Methyl *p*-formylbenzoate, anhydrous 1,2,4-trichlorobenzene (TCB), were purchased from J&K Scientific and used as received. Anhydrous CH_2Cl_2 was distilled from calcium hydride and H_2O were obtained from Milli-Q integral. Sodium [(cyclopentadienyl)tris(di(methyl-d_3)phosphito) cobaltate] (NaL_{OCD3}) (D atom > 99%)[1,2], **F-Yb-1**[3] and **H-L**[4] was synthesized according to literature methods.

Theoretical calculations

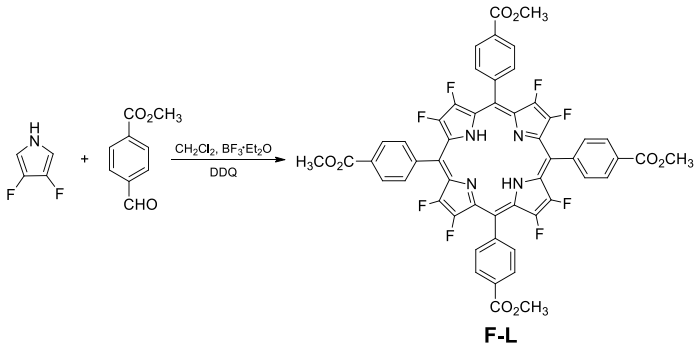
We performed single point energy calculation in the crystal structure with hybrid density functional, B3LYP,[5,6] using the program package Gaussian 09 (Revision E.01)[7]. The 6-31G(d) basis set[8,9] was used for all atoms except Yb^{3+} and Co^{2+} atoms, which was described by the Stuttgart relativistic pseudopotential and its accompanying basis set (ECP59MWB)[10,11] and the effective core potential (ECP) Lanl2DZ basis set[12].

Synthesis of F-Ln (Ln = Yb, Gd)



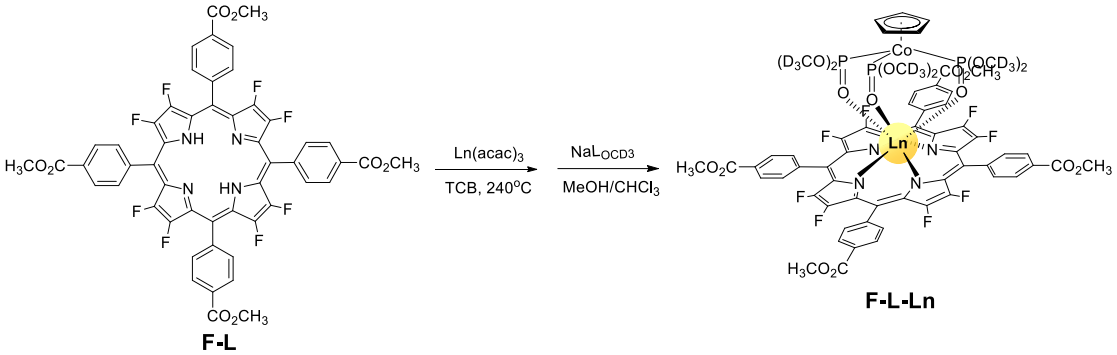
Scheme S1. Synthetic procedure of **F-Ln**.

(1) Synthesis of 2,3,7,8,12,13,17,18-octafluoro-5,10,15,20-tetrakis[4-(methyl formyl)-phenyl]porphyrin (**F-L**).



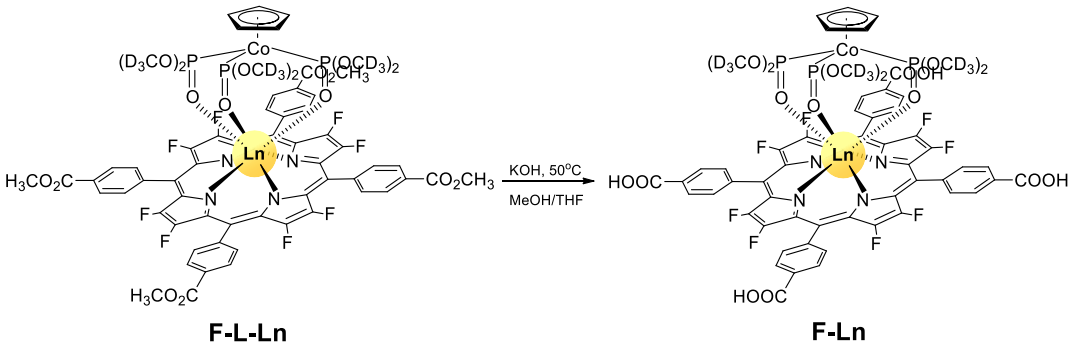
2,6,-Difluoropyrrole (103 mg, 1.0 mmol) and methyl *p*-formylbenzoate (164 mg, 1.0 mmol) were dissolved in 250 mL distilled methylene chloride. The mixture was stirred for 10 min after which a few drops of $BF_3{\cdot}Et_2O$ was added. The mixture was then stirred at room temperature for 1 h. 2,3-Dicyano-5,6-dichlorobenzoquinone (DDQ, 0.23 g, 1.0 mmol) was added. After reaction overnight at room temperature, silica gel (2g) was added to the dark solution and all solvent was evaporated. The absorbed products were placed on the top of a silica gel column. The product was obtained by using ethyl acetate as eluent (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ -4.21 (s, 2H), 4.08 (s, 12H), 8.12 (d, 4H), 8.39 (d, 4H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -139.81 (s, 4F), -145.28 (s, 4F). HR-MS (ESI^+) *m/z* $[M+H]^+$: Calcd for $C_{52}H_{31}F_8N_4O_8$ 991.2014; found: 991.1995. UV/Vis (CH_2Cl_2, 25 ℃): λ_{max}(nm) (log ε): 419 (5.69), 515 (4.45), 549 (4.43), 645(4.38).

(2) Synthesis of **F-L-Ln** (Ln = Yb, Gd).



F-L (200 mg, 0.2 mmol), $Ln(acac)_3 \cdot 3H_2O$ (500 mg, 1 mmol) and 1,2,4-trichlorobenzene (TCB) were added to a Schlenk tube and refluxed overnight. After cooling to room temperature, the reaction mixtures were transferred to a silica column, TCB was first eluted by petroleum ether, and then the unreacted free base ligand was eluted by CH_2Cl_2, corresponding Yb^{3+} complex was obtained by using CH_2Cl_2/MeOH (v/v = 5:1) as eluent and used directly to the next step. The obtained Ln complexes (acac as ancillary ligand) and 3 equiv. NaL_{OCD3} were dissolved in mixing solvent of $CHCl_3$/CH_3OH (v/v = 1:1, 5 mL). The mixture was refluxed for 8 h. After cooling to room temperature, the reaction mixtures were transferred to a silica column, **F-L-Ln(III)-L_{OCD3}** (**F-L-Ln**) was obtained by using ethyl acetate/petroleum ether (v/v = 1:2) as eluent. Complex **F-L-Yb**: Yield: 90%; ^{1}H NMR (400 MHz, $CDCl_3$) δ -5.21 (s, 5H), 4.89 (s, 12H), 9.00 (s, 4H), 9.67 (s, 4H), 11.43 (s, 4H), 17.24 (s, 4H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -131.53 (d, 8F). HR-MS (ESI^+) *m/z* $[M+H]^+$: Calcd for $C_{63}H_{34}D_{18}CoF_8N_4O_{17}P_3Yb$ 1632.2263; found: 1632.2319. UV/Vis (CH_2Cl_2, 25 ℃): λ_{max}(nm) (log ε): 409 (5.45), 550 (4.31), 591 (4.30). Complex **F-L-Gd**: Yield: 85%; HR-MS (ESI^+) *m/z* $[M+H]^+$: Calcd for $C_{63}H_{34}D_{18}CoF_8GdN_4O_{17}P_3$ 1616.2115; found: 1616.2160; elemental analysis calcd (%) for $C_{63}H_{33}D_{18}CoF_8GdN_4O_{17}P_3$: C, 46.84; H, 4.30; N, 3.47; found: C 46.55, H 4.61, N: 3.48; UV/Vis (CH_2Cl_2, 25 ℃): λ_{max}(nm) (log ε): 409 (5.47), 550 (4.35), 591 (4.31).

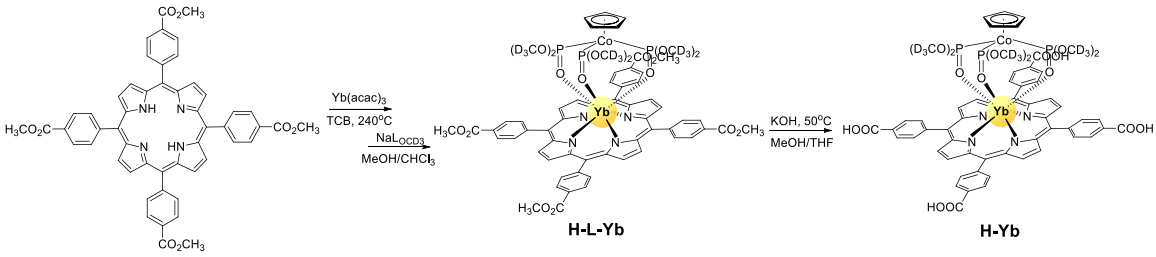
(3) Synthesis of **F-Yb** (Ln = Yb, Gd).



F-L-Ln was dissolved in mixing solvent of tetrahydrofuran/CH_3OH (v/v = 1:1, 10 mL), with the addition of 1 mL 10% KOH solution. The reaction mixtures were

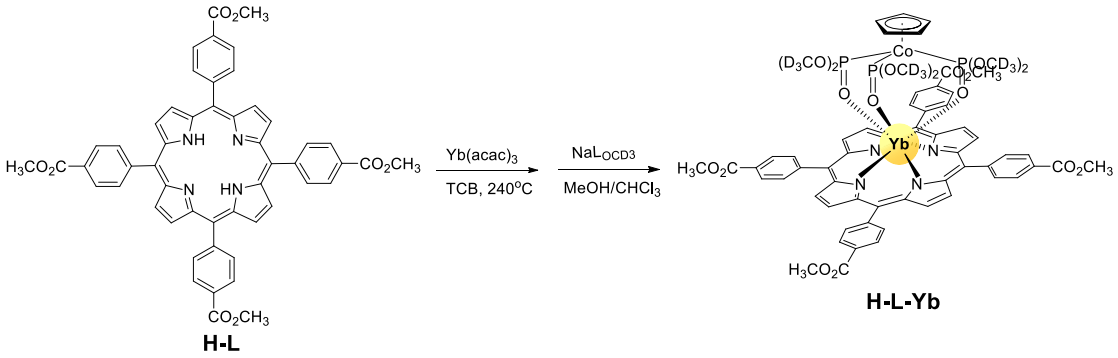
stirred at 50 °C overnight and the solution was neutralized with 0.2 M HCl. After evaporating of the organic solvent, the product **F-Ln** was obtained by centrifugalization and further recrystallization from CH_3OH. Complex **F-Yb**: Yield: 99%; ^1H NMR (400 MHz, D_6-DMSO) δ -5.45 (s, 5H), 9.39 (s, 4H), 9.69 (s, 4H), 11.45 (s, 4H), 14.32 (s, 4H), 17.46 (s, 4H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -132.71 (s, 8F). HR-MS (ESI^+) *m/z* $[M+H]^+$: Calcd for $C_{59}H_{26}D_{18}CoF_8N_4O_{17}P_3Yb$ 1576.1637; found: 1576.1631. UV/Vis (DMSO, 25 ℃): λ_{max}(nm) (log ε): 409 (5.43), 550 (4.34), 591 (4.29). Complex **F-Gd**: Yield: 95%; HR-MS (ESI^+) *m/z* [M]: Calcd for $C_{59}H_{25}D_{18}CoF_8N_4O_{17}P_3Gd$ 1559.1411; found: 1559.1441; elemental analysis calcd (%) for $C_{59}H_{25}D_{18}CoF_8N_4O_{17}P_3Gd$: C, 45.45; H, 3.94; N, 3.59; found: C 45.30, H 4.12, N: 3.51; UV/Vis (H_2O, 25 ℃): λ_{max}(nm) (log ε): 409 (5.51), 550 (4.33), 591 (4.30).

Synthesis of H-Yb



Scheme S2. Synthetic procedure of **H-Yb**.

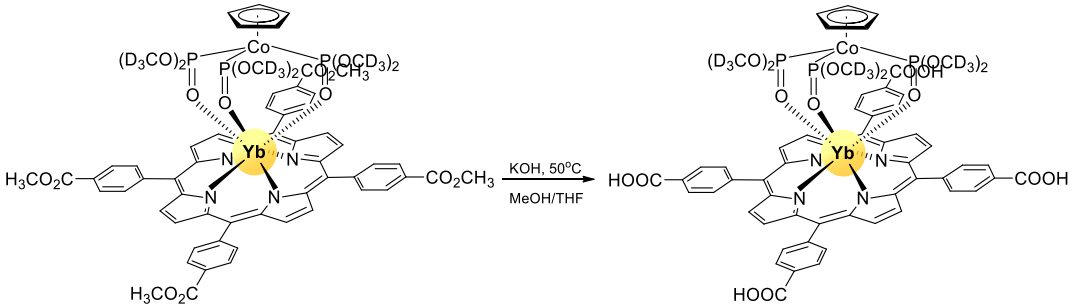
(1) Synthesis of **H-L-Yb**



H-L (200 mg, 0.2 mmol), $Yb(acac)_3•3H_2O$ (500 mg, 1 mmol) and 1,2,4-trichlorobenzene (TCB) were added to a Schlenk tube and refluxed overnight. After cooling to room temperature, the reaction mixtures were transferred to a silica column, TCB was first eluted by petroleum ether, and then the unreacted free base ligand was eluted by CH_2Cl_2, corresponding Yb^{3+} complex was obtained by using CH_2Cl_2/MeOH (v/v = 5:1) as eluent and used directly to the next step. The obtained Yb^{3+} complexes (acac as ancillary ligand) and 3 equiv. NaL_{OCD3} were dissolved in mixing solvent of $CHCl_3$/CH_3OH (v/v = 1:1, 5 mL). The mixture was refluxed for 8 h. After cooling to room temperature, the reaction mixtures were transferred to a silica

column, **H-L-Yb(III)-L_{OCD3}** (**H-L-Yb**) was obtained by using ethyl acetate/petroleum ether (v/v = 1:2) as eluent. Complex **H-L-Yb**: Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ -4.64 (s, 5H), 4.84 (s, 12H), 8.71 (s, 4H), 9.47 (s, 4H), 11.29 (s, 4H), 15.28 (s, 8H), 17.05(s, 4H). HR-MS (ESI^+) *m/z* $[M+H]^+$: Calcd for $C_{63}H_{41}D_{18}CoN_4O_{17}P_3Yb$ 1487.2938; found: 1487.2982. UV/Vis (CH_2Cl_2, 25 ℃): λ_{max}(nm) (log ε): 425 (5.42), 555 (4.30), 597 (4.28).

(2) Synthesis of **H-Yb**



H-L-Yb **H-Yb**

H-L-Yb was dissolved in mixing solvent of tetrahydrofuran/CH_3OH (v/v = 1:1, 10 mL), with the addition of 1 mL 10% KOH solution. The reaction mixtures were stirred at 50 ^{o}C overnight and the solution was neutralized with 0.2 M HCl. After evaporating of the organic solvent, the product **H-Yb** was obtained by centrifugalization and further recrystallization from CH_3OH. Complex **H-Yb**: Yield: 99%; ^{1}H NMR (400 MHz, CD_3OD) δ -5.53 (s, 5H), 8.56 (s, 4H), 8.89 (s, 4H), 9.57 (s, 4H), 11.29 (s, 4H), 15.45 (s, 8H), 16.91(s, 4H). HR-MS (ESI^+) *m/z* $[M+H]^+$: Calcd for $C_{59}H_{33}D_{18}CoN_4O_{17}P_3Yb$ 1431.2312; found: $[M-4H]^{4+}$ 356.8017. UV/Vis (H_2O, 25 ℃): λ_{max}(nm) (log ε): 426 (5.39), 556 (4.25), 597 (4.21).

Supporting figures

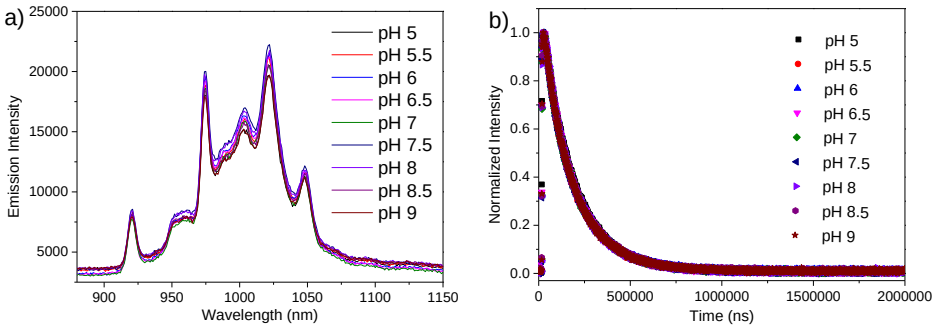


Fig. S1 a) Emission and b) decay curves monitored at 980 nm of **F-L-Yb** measured at different pH from pH 9 to pH 5 in the PBS buffer (λ_{ex} = 420 nm, $A_{420\ nm}$ = 0.1).

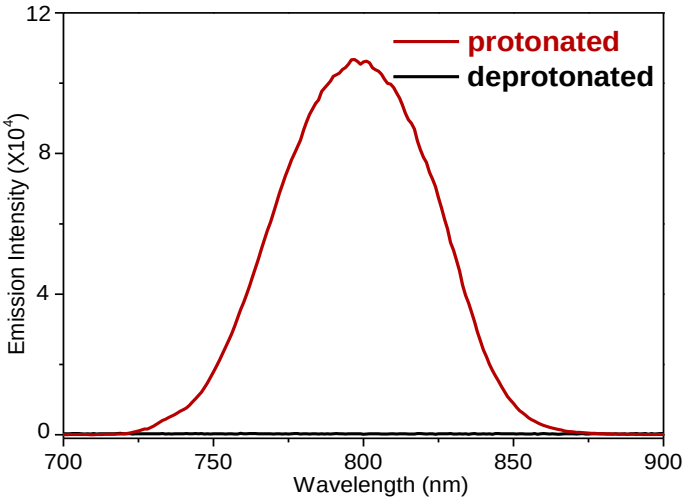


Fig. S2 Phosphorescence spectra of protonated and deprotonated **F-Gd**. The solvent is MeOH/EtOH 1:1, v/v at 77 K (λ_{ex} = 410 nm, $OD_{410\ nm}$ = 0.1). For the protonated one, **F-Gd** was directly dissolved in MeOH/EtOH. For the deprotonated one, **F-Gd** was first dissolved in H_2O, with the addition of 0.1 mM NaOH to reach a final pH value of ca. 11. Then the solution was freeze-dried and the complex was re-dissolved in MeOH/EtOH.

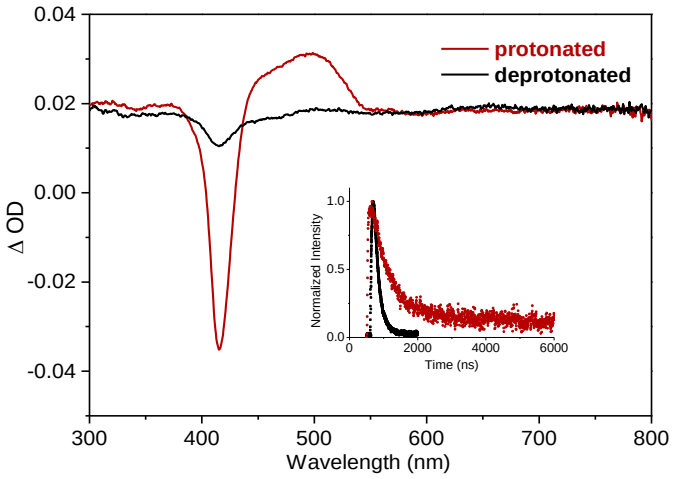


Fig. S3 Transient absorption spectra of protonated and deprotonated **F-Gd**. The spectra was measured in degassed MeOH ($OD_{410\ nm}$ = 0.05) using the 410 nm OPO laser excitation pulse (10 Hz, 2 mJ $pulse^{-1}$). Inset: triplet decay lifetime monitored at 500 nm.

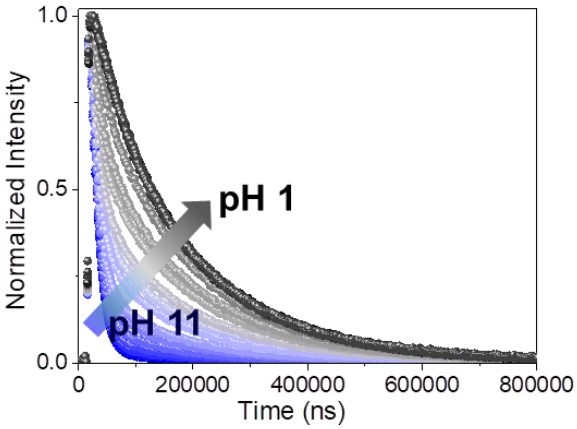


Fig. S4 Decay curves of **F-Yb** at different pH monitored at 980 nm from pH 11 to pH 1 in the Britton-Robison buffer.

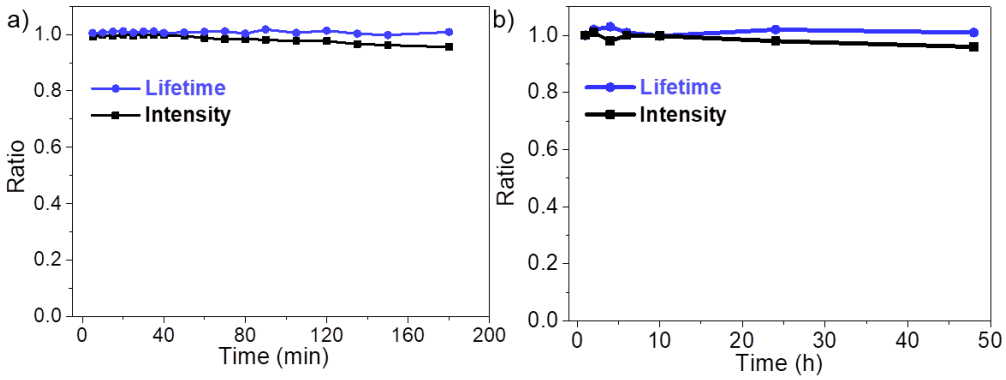


Fig. S5 Spectroscopic and lifetime evaluation of **F-Yb** at pH 1.5 KCl/HCl buffer a) with or b) without the irradiation of a 405 nm laser (0.2 W/cm^2). Emission intensity and lifetime values were referenced to the emission intensity or lifetime of Yb^{3+} (980 nm) at 0 h.

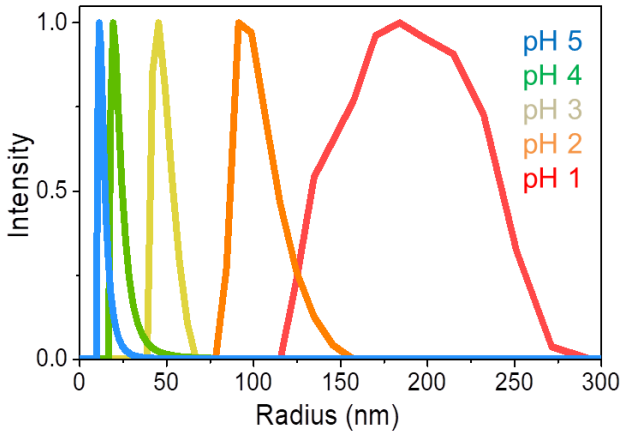


Fig. S6 Dynamic light scattering of **F-Yb** (1 μM) at different pH Britton-Robison buffer.

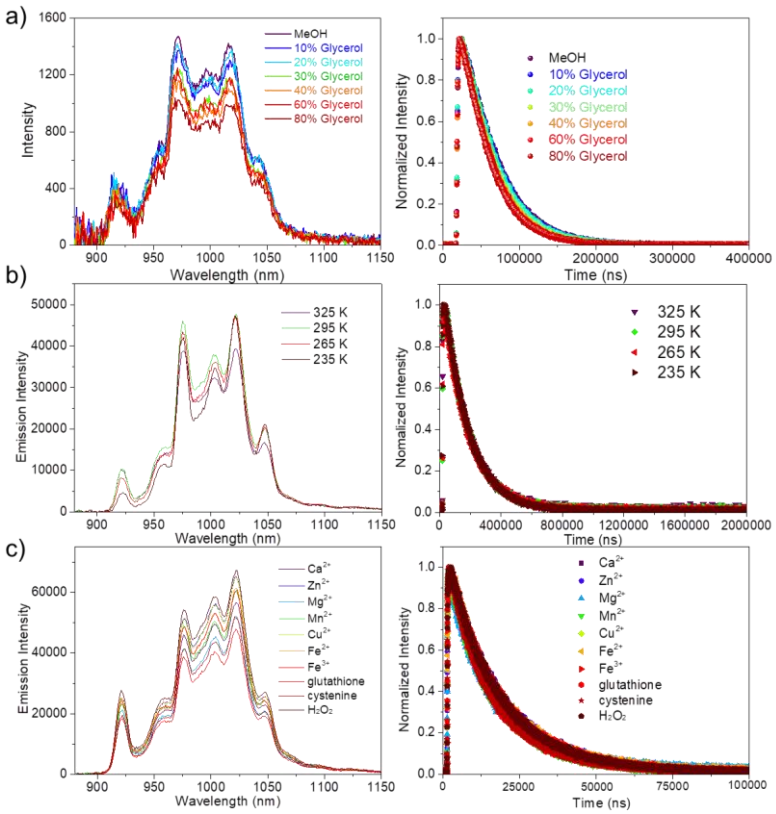


Fig. S7 Influence of the a) viscosity (measured in MeOH/Glycerol mixture), b) temperature (2-CH_3-THF solution) and c) metal ions (40 μM, pH 7.4 PBS buffer) on the luminescence (left column) and decay curve (right column,) of **F-Yb** (1 μM)**.** The decay curves were monitored at 980 nm under the excitation of 410 nm.

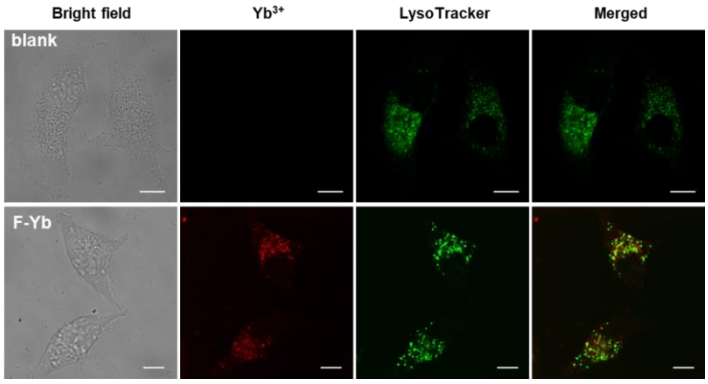


Fig. S8 Colocalization of **F-Yb** with LysoTracker Green. The NIR confocal images were performed on HeLa cells incubated with 10 μM of **F-Yb** for 4 h followed by 30 min incubation with 75 nM LysoTracker Green. a) Bright field; b) NIR signal arising from Yb^{3+} in channel 1 (λ_{ex}, 408 nm; λ_{em}, 935/170 nm bandpass); c) visible signal arising from LysoTracker Green in channel 2 (λ_{ex}, 470 nm; λ_{em}, 530/43 nm bandpass); d) merged b and c (P = 0.93). Scale bar: 10 μm.

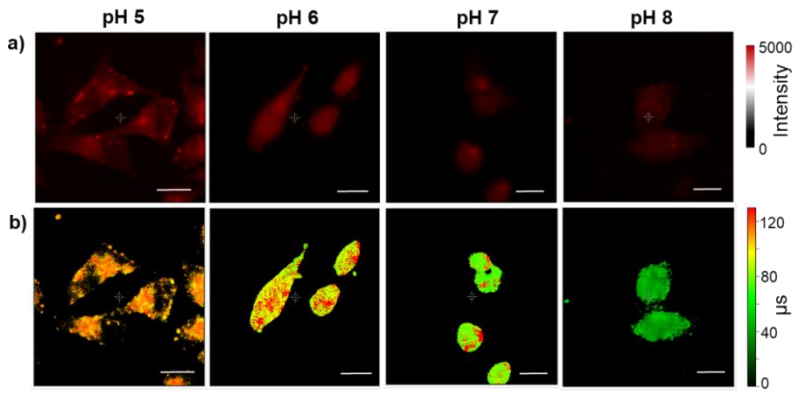


Fig. S9 The **F-Yb** images intracellular pH values with incubation of nigericin. a) NIR confocal fluorescence images and b) time-resolved fluorescence lifetime images of **F-Yb** (10 μM, 4 h) clamped at pH 5.0, 6.0, 7.0, 8.0 in HeLa cells in the presence of 10 μM nigericin (λ_{ex}, 408 nm; λ_{em}, 935/170 nm bandpass; dwell time, 1 ms). Scale bar: 10 μm.

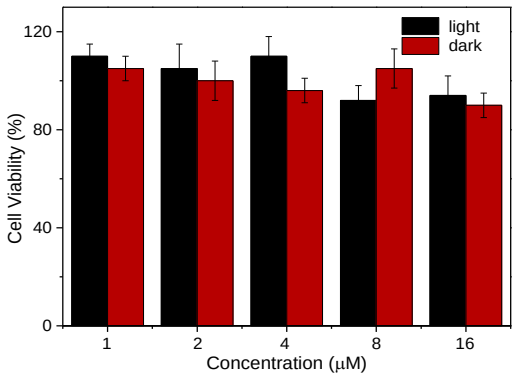
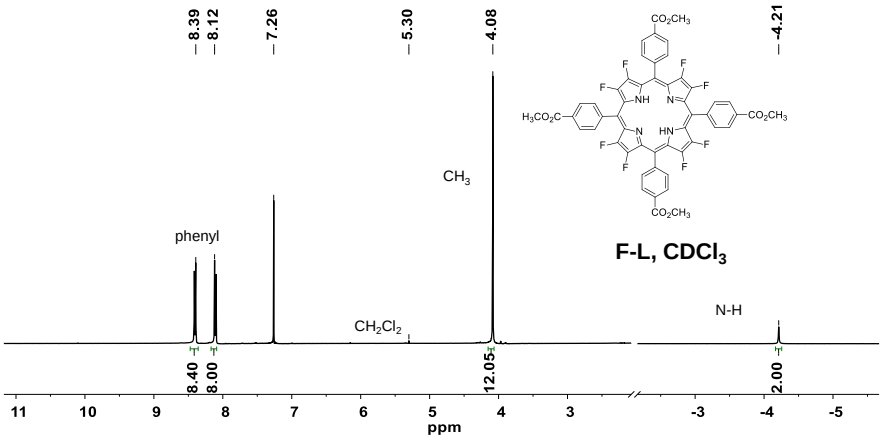
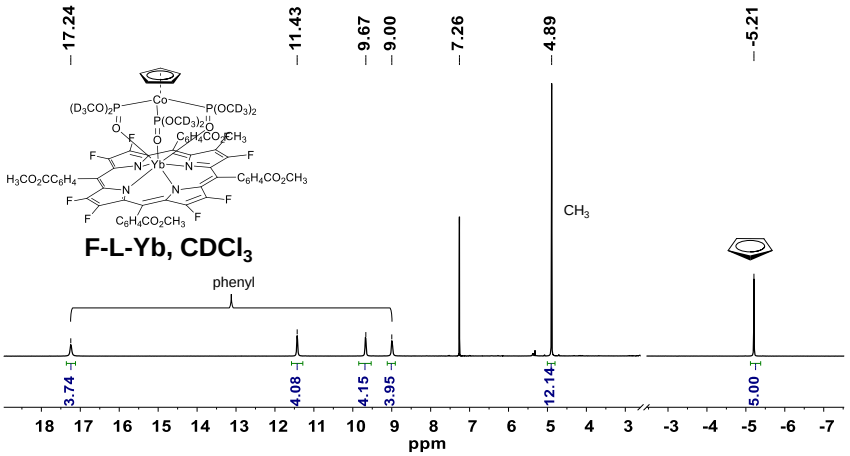
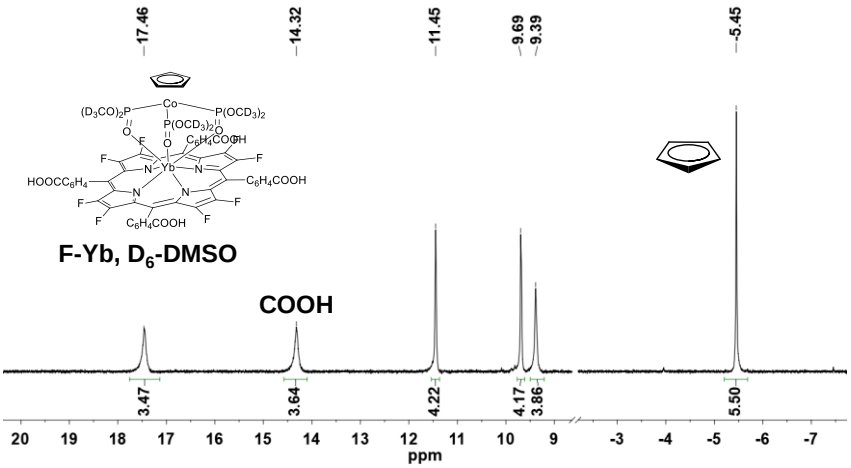


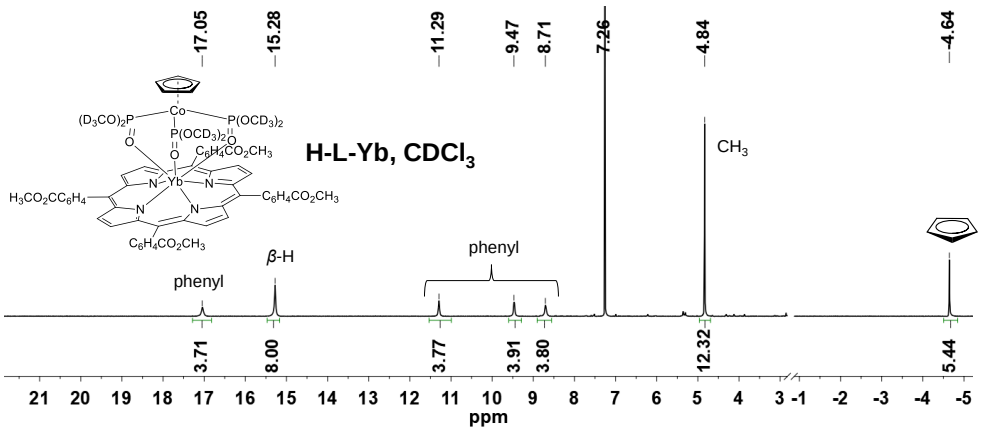
Fig. S10 Cytotoxicity of **F-Yb**. Cells were incubated with varying concentrations from 0 to 16 μM of the complex for 24 h in the dark while wells containing no cells were set as the controls. After washing three times with PBS, the cells were irradiated for 30 min in 100 mL PBS under the light irradiation (400–700 nm, 6.5 mW/cm^2) for 30 min (this step was for the photocytotoxicity). Then, PBS was replaced by 200 mL of fresh culture media. After culture for 24 h, the cells were washed three times with PBS. Then, 10 μL Cell Counting Kit-8 (CCK-8) solution and 90 μL PBS were added per well. After 2 h, the absorbance at 450 nm was read bya 96-well plate reader. The viability of HeLa cells was calculated by CV = $(A_s-A_b)/(A_c-A_b)*100\%$, where CV stands for the viability of cells, A_s, A_c, and A_b stand for the absorbance of cells containing the studied complexes, cell control (no treated cells), and blank control (wells containing neither cells nor the studied complexes), respectively.

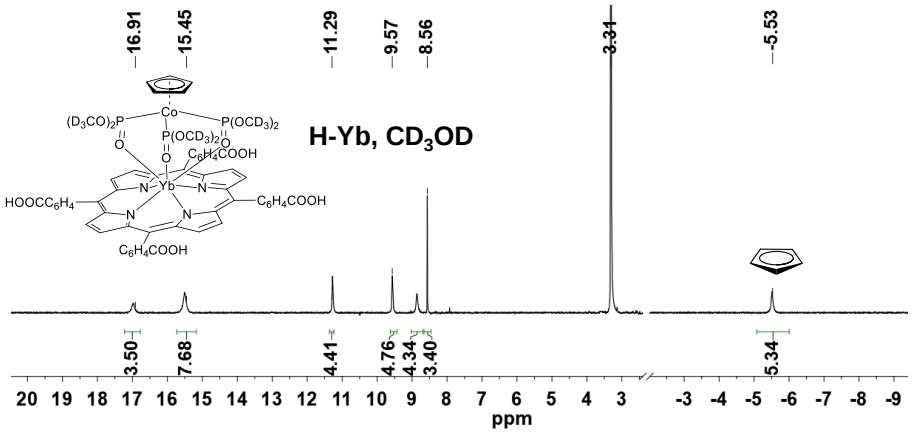
^{1}H NMR spectra



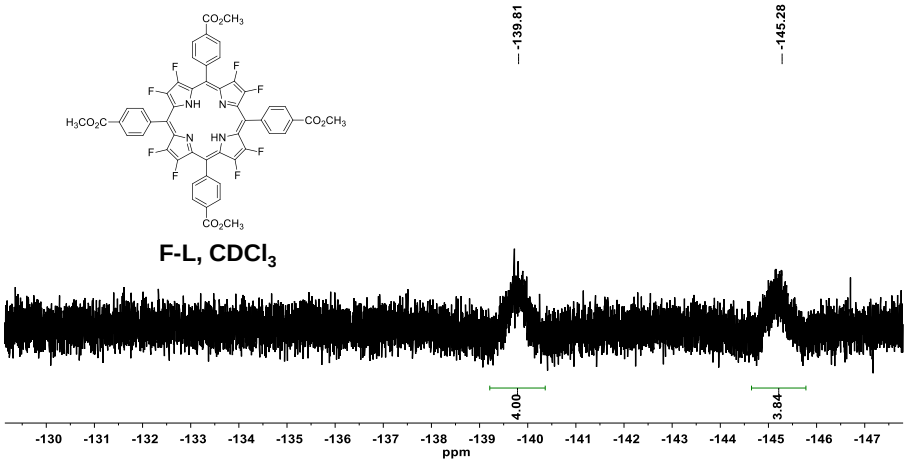


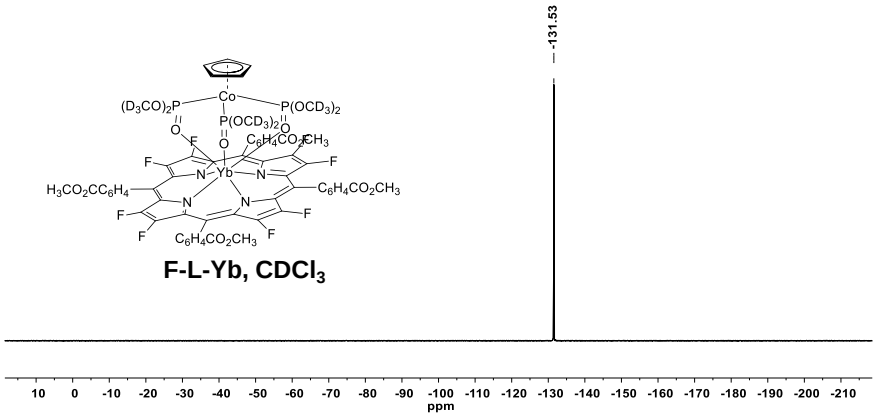


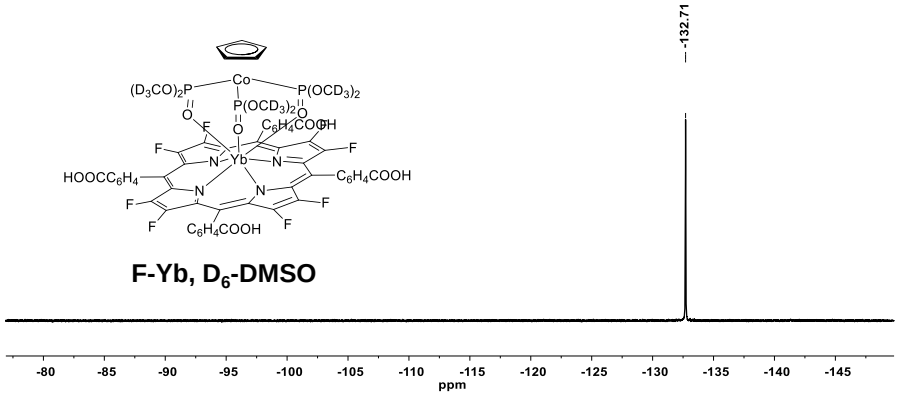




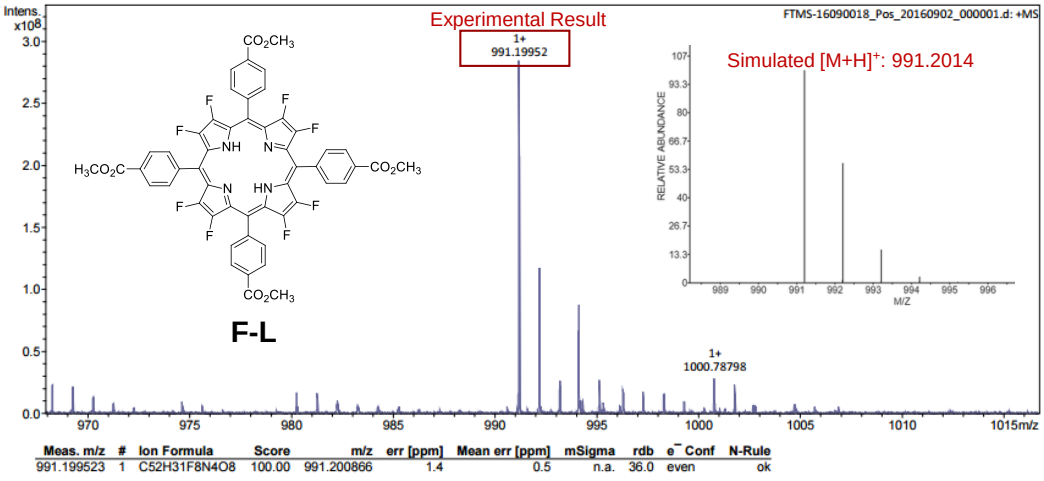
^{19}F NMR spectra

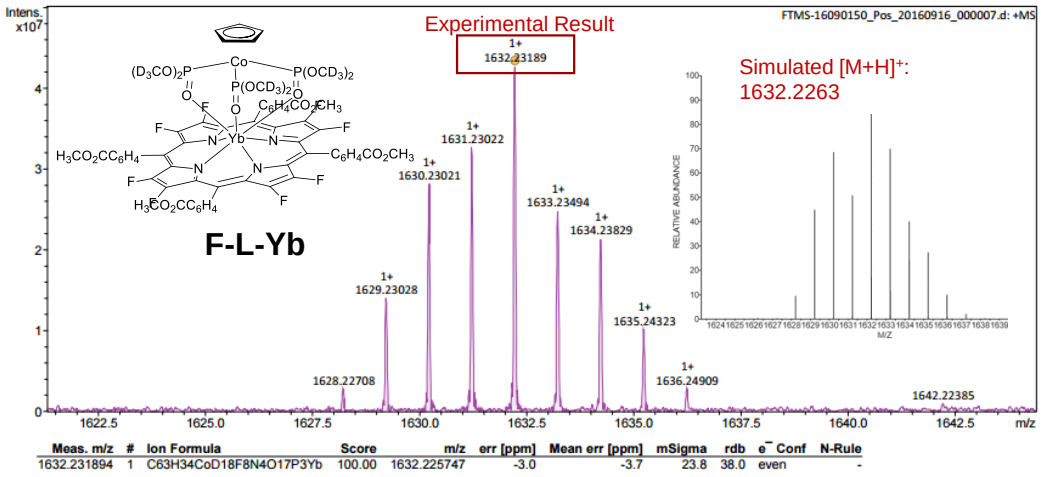



-131.53
F-L-Yb, CDCl3
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
ppm

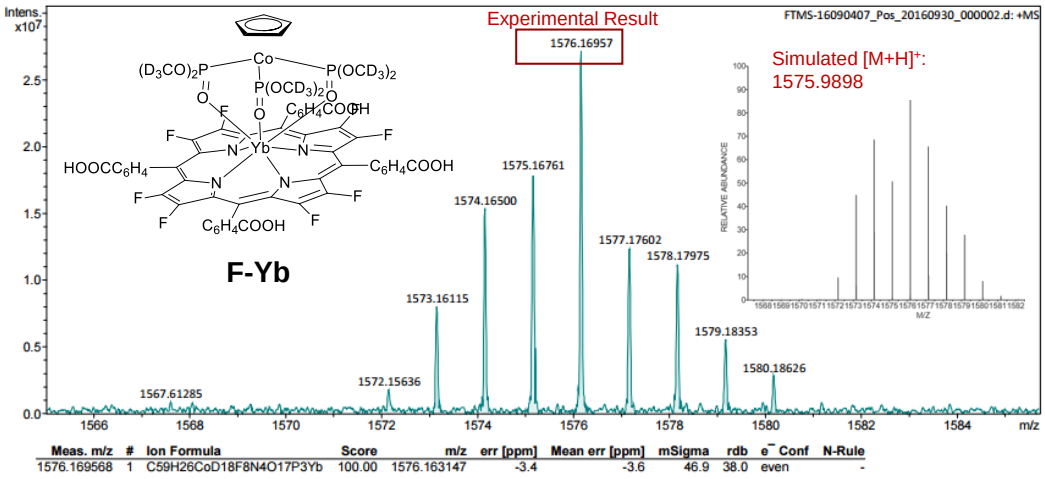

-132.71
F-Yb, D6-DMSO
-80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145
ppm

MS spectra

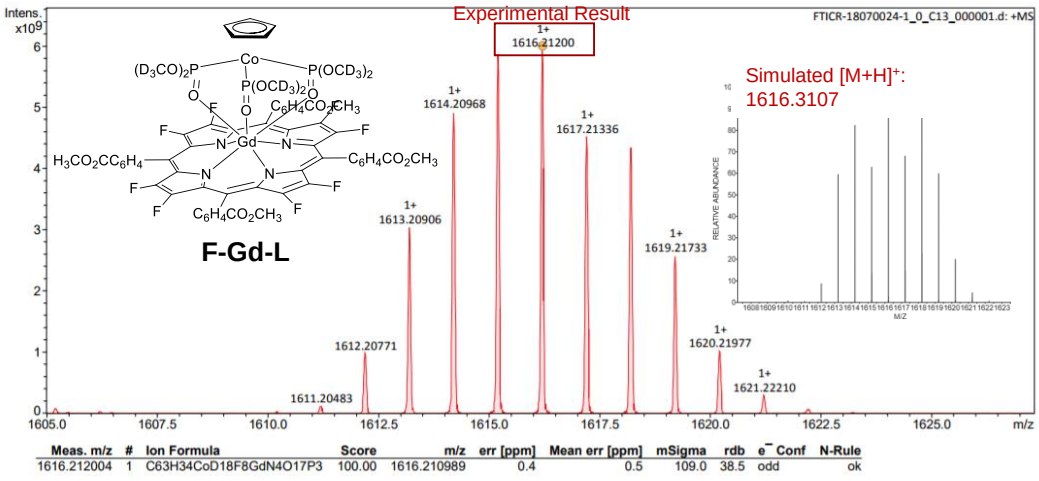

Experimental Result
1+
991.19952
FTMS-16090018_Pos_20160902_000001.d: +MS
Simulated [M+H]+: 991.2014
F-L
1+
1000.78798
Meas. m/z # Ion Formula Score m/z err [ppm] Mean err [ppm] mSigma rdb e- Conf N-Rule
991.199523 1 C52H31F8N4O8 100.00 991.200866 1.4 0.5 n.a. 36.0 even ok



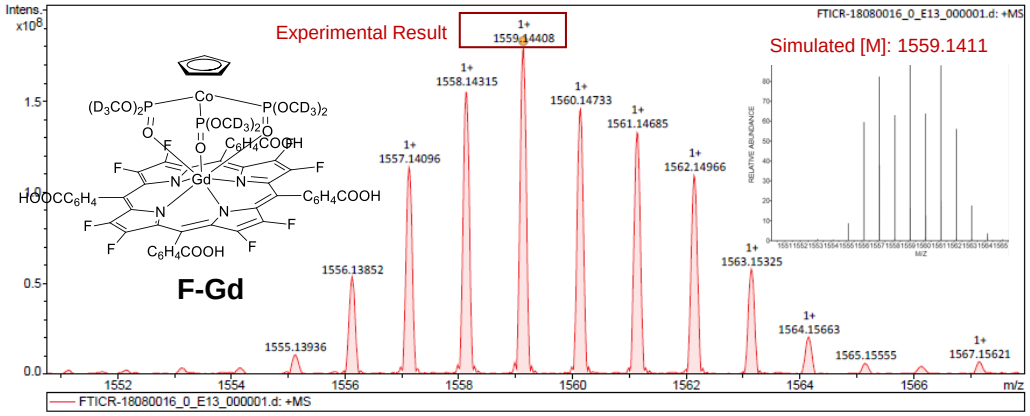
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1632.231894	1	C63H34CoD18F8N4O17P3Yb	100.00	1632.225747	-3.0	-3.7	23.8	38.0	even	-



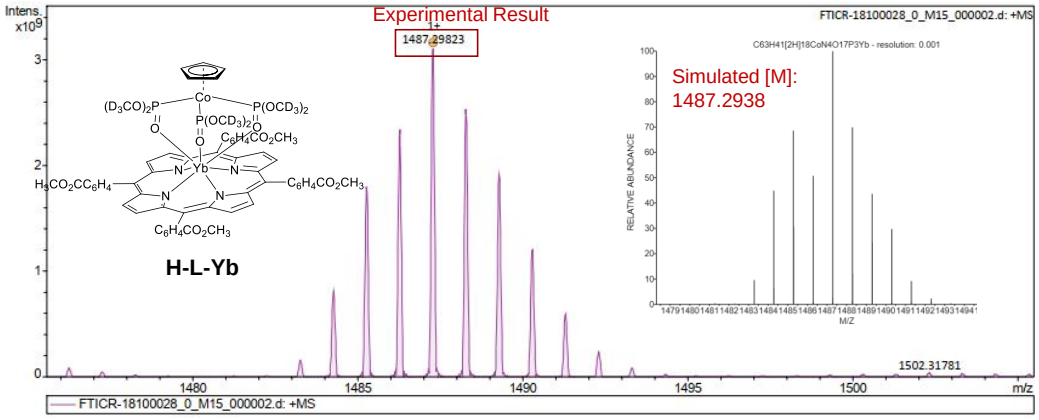
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1576.169568	1	C59H26CoD18F8N4O17P3Yb	100.00	1576.163147	-3.4	-3.6	46.9	38.0	even	-



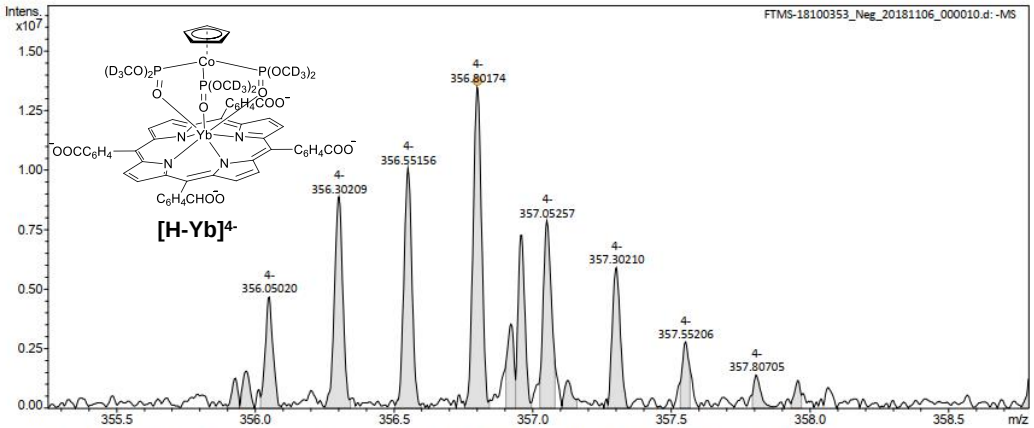
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1616.212004	1	C63H34CoD18F8GdN4O17P3	100.00	1616.210989	0.4	0.5	109.0	38.5	odd	ok



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1559.144079	1	C59H25CoD18F8GdN4O17P3	100.00	1559.140563	-1.3	-1.3	136.8	39.0	even	ok

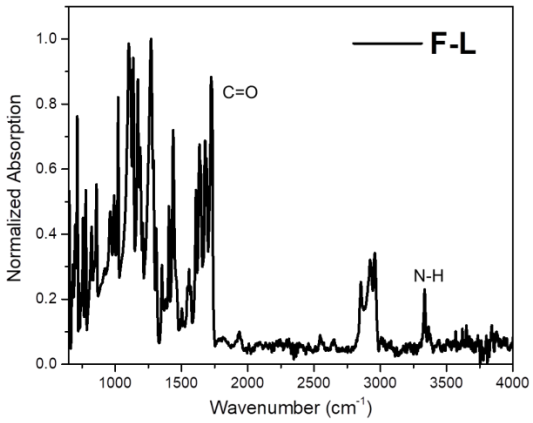


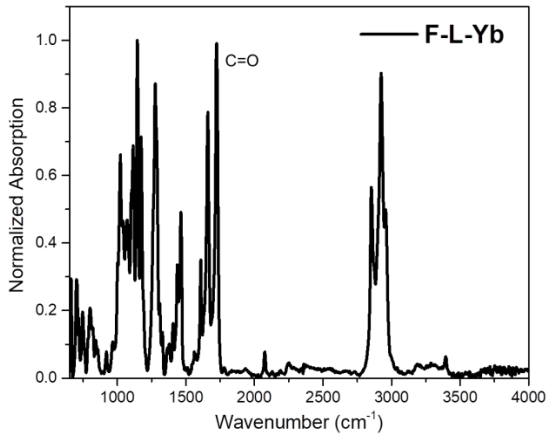
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1487.298234	1	C63H41CoD18N4O17P3Yb	100.00	1487.293296	-2.5	-3.1	113.5	38.5	odd	-

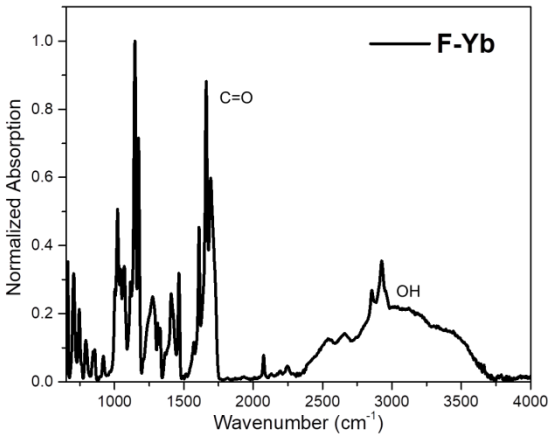


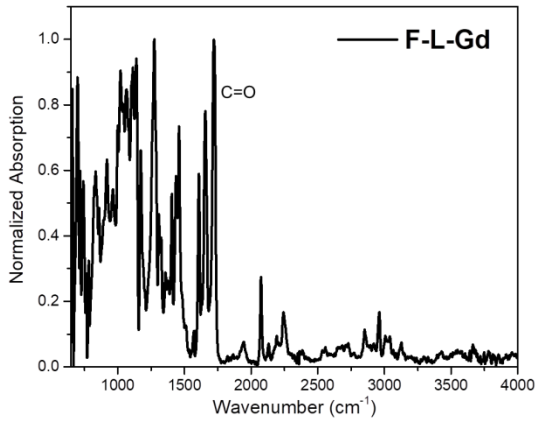
Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
356.801739	1	C59H33CoD16N4O17P3Yb	100.00	356.801310	-0.4	-0.7	30.8	41.0	even	-
	2	C59H29CoD18N4O17P3Yb	68.41	356.800536	-2.6	-2.6	30.8	42.0	even	-

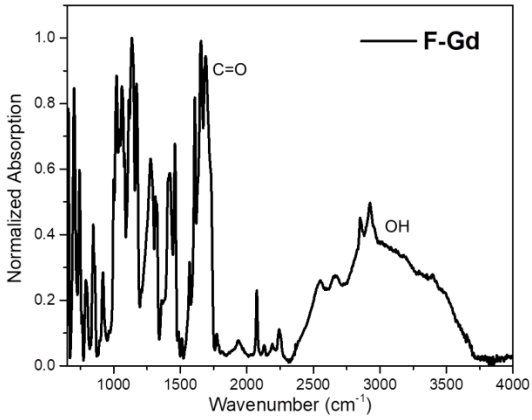
IR spectra

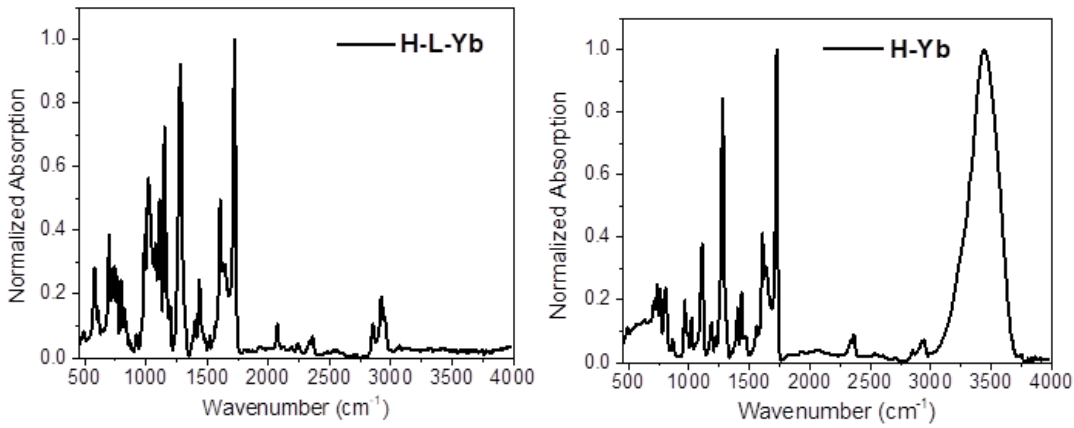

F-L
C=O
N-H
Normalized Absorption
Wavenumber (cm-1)


F-L-Yb
C=O
Normalized Absorption
Wavenumber (cm-1)


F-Yb
C=O
OH
Normalized Absorption
Wavenumber (cm-1)


F-L-Gd
C=O
Normalized Absorption
Wavenumber (cm-1)


F-Gd
C=O
OH
Normalized Absorption
Wavenumber (cm-1)



References

1. W. Klaui, *Angew. Chrm. Int. Ed.*, 1990, **29**, 627-637.
2. K. J. Allen, et al., *Inorg. Chem.*, 2012, **51**, 12436-12443.
3. Y. Ning, J. Tang, Y.-W. Liu, J. Jing, Y. Sun and J.-L. Zhang, *Chem. Sci*. 2018, **9**, 3742-3753.
4. J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney, and A. M. Marguerettaz, *J. Org. Chem.* 1987, **52**, 827-836.
5. C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 1988, **37**, 785–789.
6. A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652.
7. M. J. Frisch, et al., *Gaussian 09 (Revision D.01)*, Gaussian, Inc., Wallingford CT, 2009.
8. M. M. Francl, W. J. Petro, W. J. Hehre, J. S. Binkley, M. S. Gordon, D. J. DeFree and J. A. Pople, *J. Chem. Phys.*, 1982, **77**, 3654.
9. P. C. Hariharan and J. A. Pople, *Theor. Chem. Acc.*, 1978, **28**, 213.
10. D. Andrae, U. Haeussermann, M. Dolg, H. Stoll and H. Preuss, *Theor. Chim. Acta.*, 1990, **77**, 123–141.
11. J. M. L. Martin and A. Sundermann, *J. Chem. Phys.*, 2001, **114**, 3408–3420.
12. P. J. Hay and W. R. Wadt, *J. Chem. Phys.*, 1985, **82**, 299.