Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information

Zinc(II)-Induced Control of the Internalization of a Near-Infrared Fluorescent Probe by Live Cells

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Additional Experimental Data

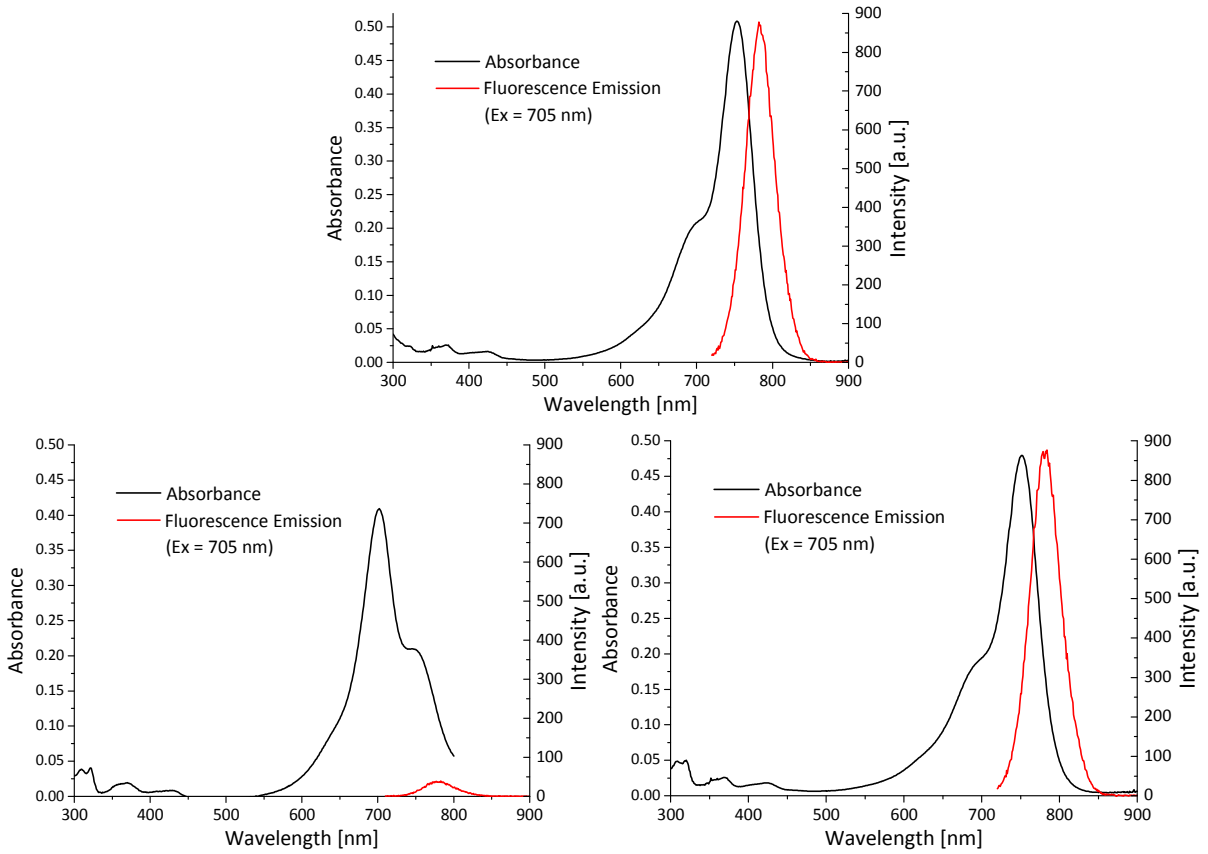


Fig. S1 - UV/Vis absorbance and fluorescence emission spectra of 2 μM solutions of **NIR-Z** (top), **(NIR-Z)$_2$Zn^{II}** (bottom, left) and **(NIR-Z)Zn^{II}** (bottom, right) in deionized H_2O.

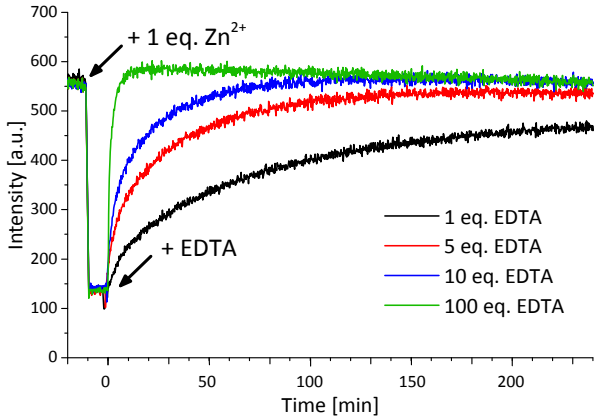


Fig. S2 - Complex stability of **(NIR-Z)$_2$Zn^{II}** in presence of EDTA. Fluorescence emission at 780 nm was monitored. One equivalent of metal was added to two equivalents of **NIR-Z** in PBS followed by the addition of different amounts of EDTA.

Dye	NIR-Z	$(NIR\text{-}Z)_2Zn^{II}$	$(NIR\text{-}Z)Zn^{II}$
partition coefficient (octan-1-ol/deion. water)	0.46	0.74	1.03
log K_{ow}	-0.34	-0.13	0.01

Fig. S3 - Partition coefficients of **NIR-Z** and its complexes. 1 μM solutions of **NIR-Z** were prepared with different metal concentrations in deionized water (2 ml) and equilibrated against octanol (8 ml) by 30 minutes of shaking in a falcon tube. After phase separation, the dye concentration in the aqueous phase was determined by absorption spectroscopy.

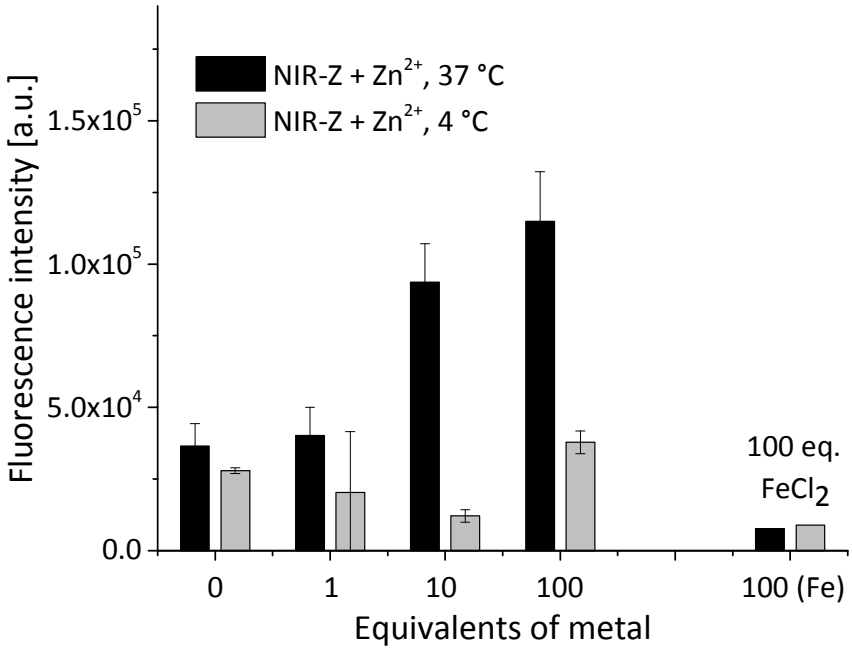


Fig. S4 - Influence of temperature on cellular uptake. Flow cytometric determination of the fluorescence intensity of live HeLa cells after 50 min of incubation with **NIR-Z** (1 μM) in PBS with increasing concentrations of zinc(II) at 37 °C (black bars) or 4 °C (grey bars).

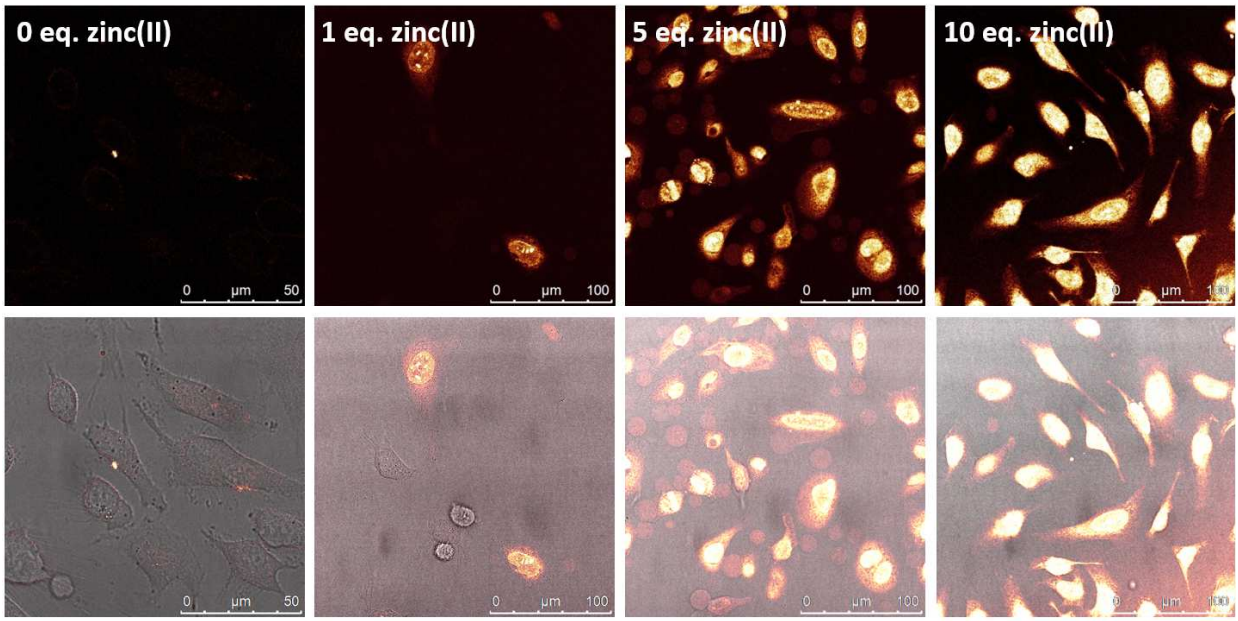


Fig. S5 - Representative confocal fluorescence microscopy images (top row: fluorescence channel; bottom row: overlay with transmission images) of live HeLa cells after incubation with **NIR-Z** (10 μM) in PBS with increasing equivalents of $ZnCl_2$ for 5 minutes at 37 °C.

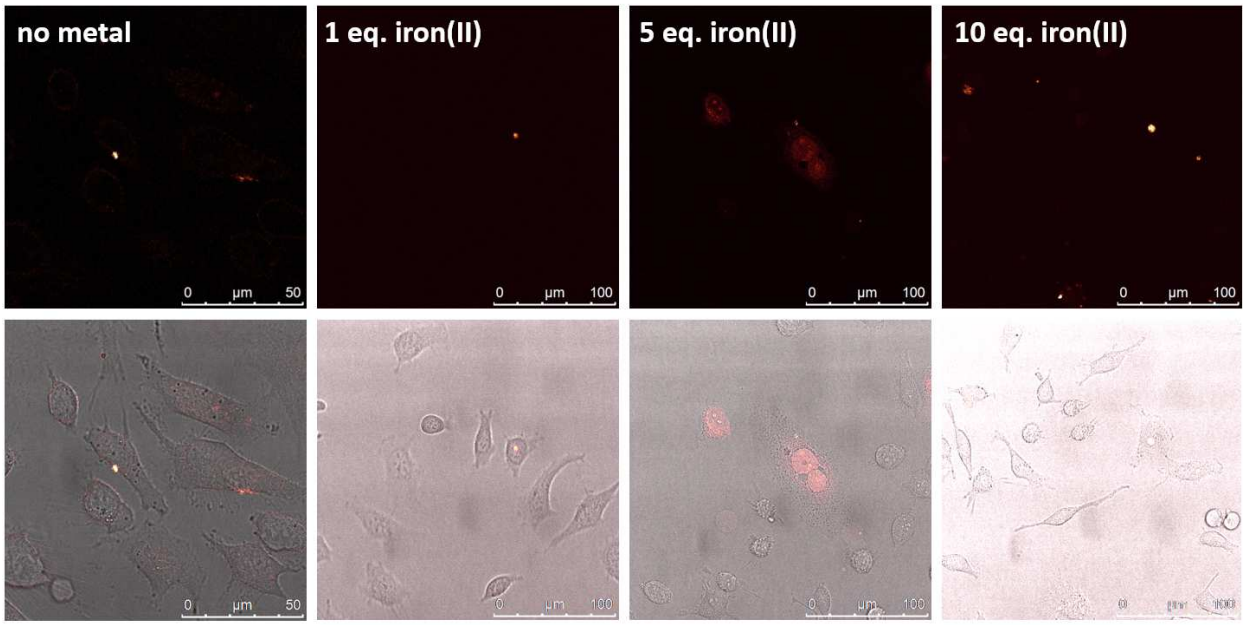


Fig. S6 - Representative confocal fluorescence microscopy images (top row: fluorescence channel; bottom row: overlay with transmission images) of live HeLa cells after incubation with **NIR-Z** (10 μM) in PBS with increasing equivalents of $FeCl_2$ for 5 minutes at 37 °C.

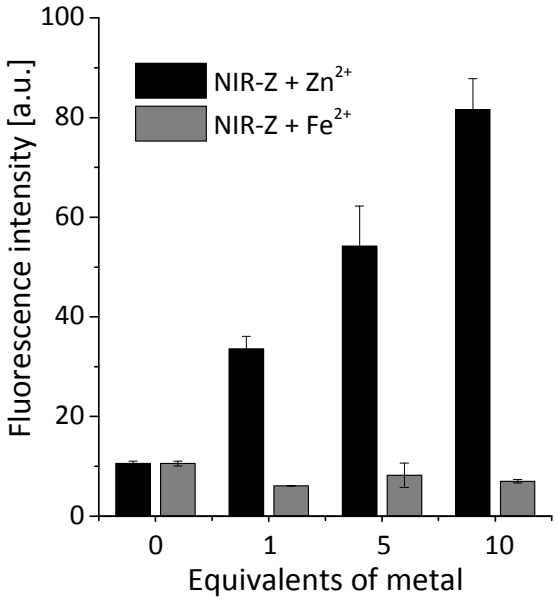


Fig. S7 - Image analysis of live HeLa cells after treatment with **NIR-Z** in the presence of $ZnCl_2$ (black) or $FeCl_2$ (grey). Fluorescence intensities were extracted using ImageJ.

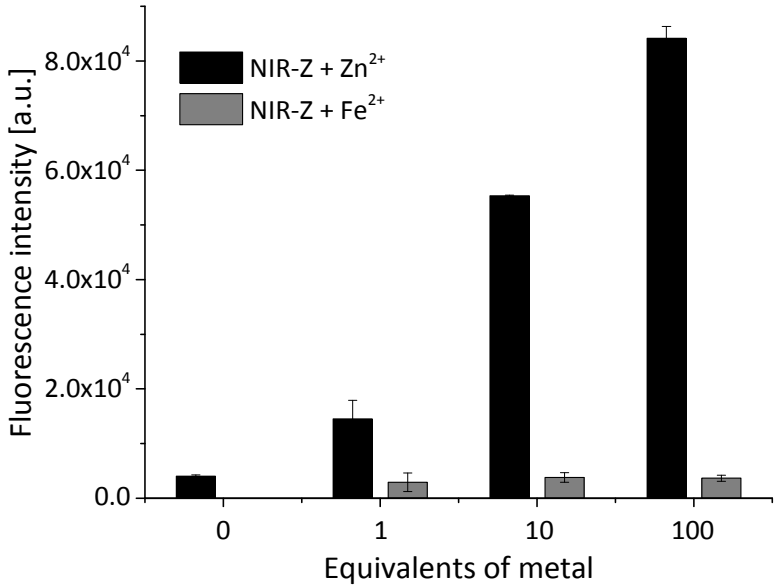


Fig. S8 - Flow cytometric determination of the fluorescence intensity of live **hTERT RPE1** cells after 50 min of incubation with **NIR-Z** (1 μM) in PBS with increasing concentrations of zinc(II) (black bars) or with increasing concentrations of iron(II) (grey bars) at 37 °C.

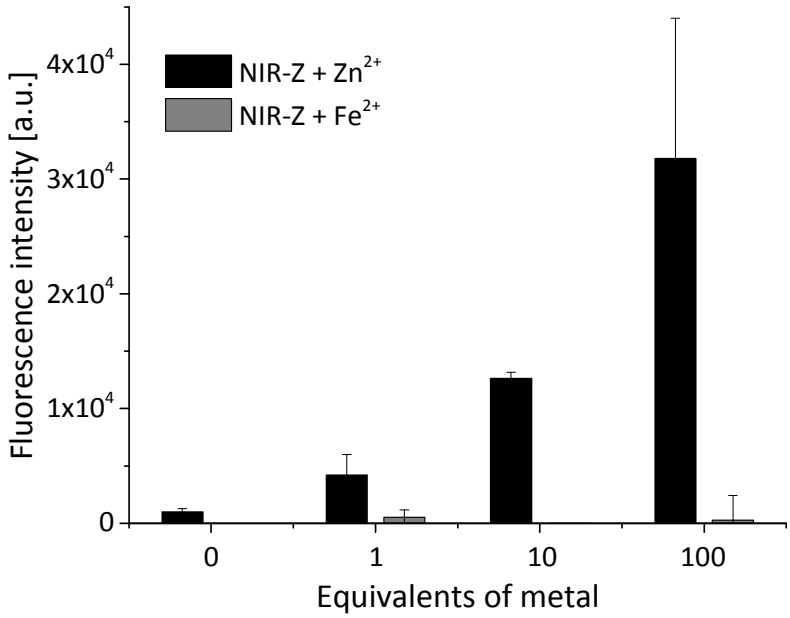


Fig. S9 - Flow cytometric determination of the fluorescence intensity of live **H1975** cells after 50 min of incubation with **NIR-Z** (1 μM) in PBS with increasing concentrations of zinc(II) (black bars) or with increasing concentrations of iron(II) (grey bars) at 37 °C.

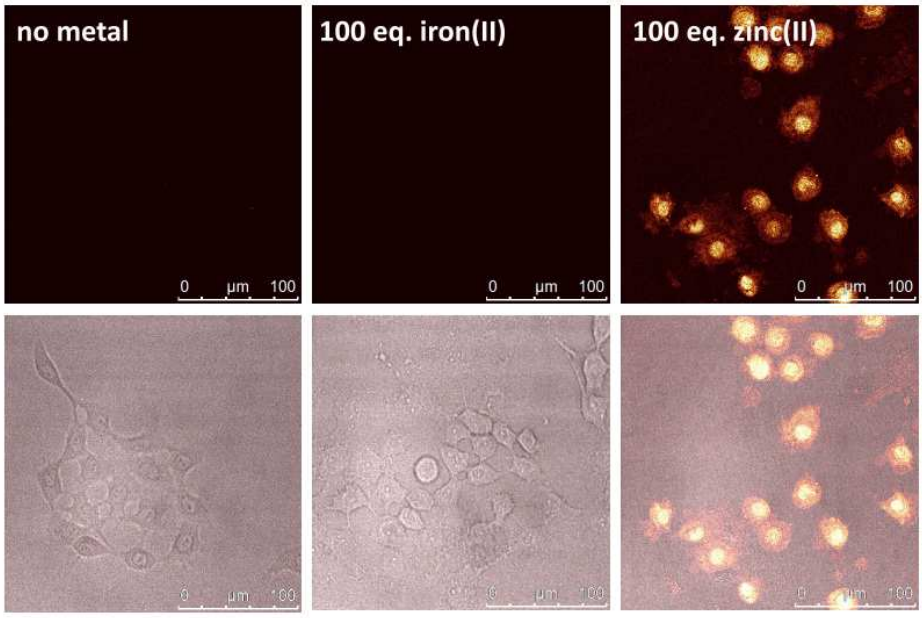


Fig. S10 - Representative confocal fluorescence microscopy images (top row: fluorescence channel; bottom row: overlay with transmission images) of live **H1975** cells after incubation with **NIR-Z** (10 μM) in PBS and **NIR-Z** (10 μM) in PBS supplemented with 100 eq. $ZnCl_2$ or $FeCl_2$ for 7 minutes at 37 °C.

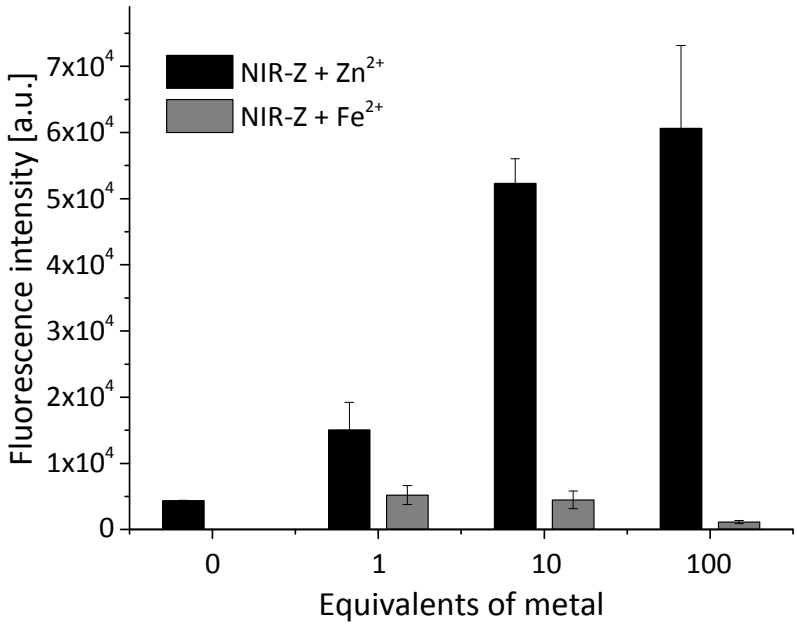


Fig. S11 - Flow cytometric determination of the fluorescence intensity of live **H1650** cells after 50 min of incubation with **NIR-Z** (1 μM) in PBS with increasing concentrations of zinc(II) (black bars) or with increasing concentrations of iron(II) (grey bars) at 37 °C.

Instruments and Methods

Air- and moisture sensitive substances were handled under a dry, inert argon atmosphere using standard Schlenk-techniques in glassware that was flame-dried prior to use. Argon was used without further drying, anhydrous solvents were obtained commercially or dried using standard techniques and stored under argon over molecular sieves. All temperature sensitive compounds were stored at 4 °C or -20 °C. Dye stock solutions were prepared in anhydrous DMSO and stored at -20 °C in the dark.
In order to improve comprehensibility, simplified names were used for some synthesized compounds rather than using exact IUPAC names. Atom numbering was done for NMR-assignments only and is not always based on the compound's name.

Chemicals used for synthesis were obtained from Sigma-Aldrich, Fluka, Riedel-de-Haën, Merck, Bachem or Roth. All substances with 98 % purity or higher were used without further purification.

Nuclear magnetic resonance spectra were recorded on Bruker Avance II (400 MHz) or Bruker Avance III (600 MHz) spectrometers. Chemical shifts δ are given in ppm and coupling constants J in Hz. All spectra were calibrated using the residual ^{1}H- or ^{13}C-signals of the deuterated solvents.[1] Spectra were recorded at 298 K. The following abbreviations were used to describe the multiplicities of the signals: s (singlet), d (doublet), t (triplet), qn (quintet), m (multiplet). Signals were assigned using DEPT, COSY and HSQC spectra.

Mass spectra were recorded on a Bruker ApexQe hybrid 9.4 T FT-ICR (HR-ESI), a JEOL JMS-700 magnetic sector (EI and HR-EI) or a Bruker Microflex (MALDI-TOF; used primarily for the identification of HPLC peaks).

Preparative and semi-preparative HPLC-purifications were performed on Shimadzu HPLC systems at 20 °C. All samples were filtered through C18 silica (Waters) prior to HPLC-purification.

The C18 columns named in line with the synthetic protocols were used as stationary phases, deionized H_2O with 0.1 % TFA and MeCN served as eluents. Products were collected manually and identified by MALDI-TOF mass spectrometry.

Analytical HPLCs for all derivatives were performed on a Shimadzu HPLC system.

UV/Vis Spectroscopic Analysis and Molar Extinction Coefficients

UV/Vis-Spectra were recorded on a Cary 100 Bio by Varian using 3 mL PMMA cuvettes by Sigma-Aldrich. Stock solutions of the dyes for the determination of molar extinction coefficients were prepared in from weighted samples in DMSO or in d_6-DMSO with 50 mM 2-propanol as standard using NMR-spectroscopy to determine the concentration of these stock solutions. The molar extinction coefficient was obtained using Beer's law at 0.1 μM to 0.5 μM dilutions in PBS or deionized water (*Trace*SELECT for trace analysis, Fluka).

Fluorescence Measurements and Quantum Yields

Fluorescence measurements were performed on a Cary Eclipse spectrophotometer by Varian. 3 mL PMMA cuvettes by Sigma-Aldrich were used for all measurements. The relative fluorescence quantum yield was determined using indocyanine green as reference following equation (1)[2]

$$\Phi_x = \Phi_{ref} \cdot \left(\frac{A_{ref}}{A_x}\right) \cdot \left(\frac{F_x}{F_{ref}}\right) \cdot \left(\frac{n_x}{n_{ref}}\right)^2 \qquad (1)$$

where Φ_x is the quantum yield of the new compound, Φ_{ref} is the quantum yield of the reference substance in DMSO, A_{ref} is the absorbance at the fluorescence excitation wavelength (705 nm) of the reference solution while A_x is the absorbance at 705 nm of the solution of the new compound, n is the refractive index of the solvents used for the measurement (DMSO for indocyanine green and PBS or deionized water (*Trace*SELECT for trace analysis, Fluka) for the new compounds) and F_x and F_{ref} are the areas under the curves of the fluorescence emission spectra for the new compounds and the reference solutions. Samples were excited at 705 nm.

Octanol partitioning

Partition coefficients were determined between octan-1-ol and deionized water following the procedure described by Licha *et al.*[3] Prior to the experiment, water was staturated with octanol and octanol was saturated with water.

A 1 μM solution of **NIR-Z** with different metal concentrations in deionized water (2 ml) was equilibrated against octanol (8 ml) by 30 minutes of shaking in a falcon tube. After phase separation, the dye concentration in the aqueous phase was determined by absorption spectroscopy. The partition coefficient was calculated according to

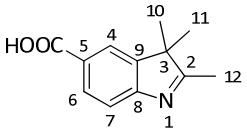
$$\frac{c(aqueous, initial) - c(aqueous, separated)}{c(aqueous,\ separated)}$$

divided by the phase volume factor of 4. Experiments were performed twice for each metal concentration.

Synthetic Procedures

5-Carboxy-2,3,3-trimethyl-3*H*-indole (I)

This is a known compound which was synthesized following a procedure by Terpetschnig *et al*.[4] The work-up was modified.



$C_{12}H_{13}NO_2$
203.24 g/mol

Hydrazinobenzoic acid (4.56 g, 30.0 mmol, 1.0 eq.), sodium acetate (5.00 g, 60.0 mmol, 2.0 eq.) and 3-methyl-butan-2-one (4.60 mL, 3.70 g, 43.5 mmol, 1.5 eq.) were dissolved in glacial acetic acid (30 mL) and stirred for 1 hour at room temperature and for 5 hours at 130 °C. Subsequently, the solvent was evaporated and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water (3 x 100 mL) and dried using anhydrous $MgSO_4$. After removing the solvent under reduced pressure, **I** was obtained as pale brown solid (3.70 g, 18.2 mmol, 61%).

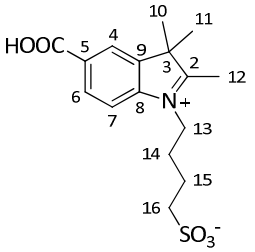
^{1}H NMR (600.13 MHz, $CDCl_3$)
δ = 1.38 (s, 6H, H-10/H-11), 2.40 (s, 3H, H-12), 7.69 (d, $^3J_{H\text{-}H}$ = 8.04 Hz, 1H, H-1), 8.07 (d, $^4J_{H\text{-}H}$ = 1.50 Hz, 1H, H-4), 8.15 (dd, $^3J_{H\text{-}H}$ = 8.05 Hz, $^4J_{H\text{-}H}$ = 1.50 Hz, 1H, H-6).

^{13}C {^{1}H} NMR (150.92 MHz, $CDCl_3$)
δ = 15.6 (1C, CH_3, C-12), 23.0 (2C, CH_3, C-10/C-11), 54.0 (1C, C_q, C-3), 119.7 (1C, CH, C-7), 123.4 (1C, CH, C-6), 127.2 (1C, C_q, C-5), 131.0 (1C, CH, C-4), 145.5 (1C, C_q, C-9), 157.0 (1C, C_q, C-8), 171.3 (1C, C_q, C-2), 192.9 (1C, C_q, COOH).

MS (HR-EI$^+$)
m/z = 203.0936 $[M+H]^+$, calculated for $C_{12}H_{13}O_2N^+$: 203.0941.

5-Carboxy-1-(4-sulfobutyl)-2,3,3-trimethyl-*3H*-indolium betaine (II)

This is a known compound which was synthesized following a procedure by Terpetschnig *et al*.[4]



$C_{16}H_{21}NO_5S$
339.41 g/mol

I (1.22 g, 6.00 mmol, 1.0 eq.) and 1,4-butanesultone (3.21 mL, 4.30 g, 35.0 mmol, 5.8 eq.) were suspended in 1,2-dichlorobenzene (20 mL) and stirred at 180 °C for 8 hours. After cooling to room

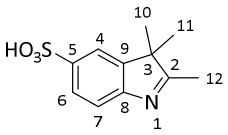
temperature, the precipitate was collected by filtration and washed with acetone. The solid material was dried *in vacuo* to yield **II** as red powder (1.85 g, 5.45 mmol, 91%).

^{1}H NMR (399.89 MHz, d_6-DMSO)
δ = 1.56 (s, 6H, H-10/H-11), 1.72 - 1.74 (m, 2H, H-15), 1.95 - 1.97 (m, 2H, H-14), 2.49 (m, 2H, H-16 (superimposed by DMSO)), 2.88 (s, 3H, H-12), 4.49 - 4.51 (m, 2H, H-13), 8.15 (br. s, 2H, H-4/H-6), 8.37 (s, 1H, H-7).

^{13}C {^{1}H} NMR (150.92 MHz, d_6-DMSO)
δ = 14.2 (1C, CH_3, C-12), 21.7 (2C, CH_3, C-10/C-11), 22.1 (1C, CH_2, C-15), 25.9 (1C, CH_2, C-14), 47.5 (1C, CH_2, C-16), 50.1 (1C, CH_2, C-13), 54.4 (1C, C_q, C-3), 115.8 (1C, CH, C-7), 124.3 (1C, CH, C-6), 130.4 (1C, CH, C-4), 131.5 (1C, C_q, C-5), 142.2 (1C, C_q, C-9), 144.3 (1C, C_q, C-8), 166.4 (1C, C_q, C-2), 199.6 (1C, C_q, COOH).

MS (HR-ESI$^-$): m/z = 338.1061 $[M-H]^-$, calculated for $C_{16}H_{20}NO_5S^-$: 338.1067.

2,3,3-Trimethyl-3*H*-indole-5-sulfonic acid (III)
This is a known compound which was synthesized following a procedure by Pham *et al*.[5] (slightly modified).



$C_{11}H_{13}NO_3S$
239.29 g/mol

Hydrazinobenzenesulfonic acid (5.00 g, 26.6 mmol, 1.0 eq.), sodium acetate (4.36 g, 53.2 mmol, 2.0 eq.) and 3-methyl-butan-2-one (8.40 mL, 6.75 g, 78.4 mmol, 3.0 eq.) were dissolved in glacial acetic acid (30 mL) and stirred at 130 °C for 5 hours under argon. After cooling to room temperature, Et_2O was added carefully to the stirring red solution until the product precipitated. The solid material was collected by filtration and dried *in vacuo* to yield **III** (5.81 g, 24.3 mmol, 91%) as pink powder.

^{1}H NMR (399.89 MHz, D_2O)
δ = 1.35 (s, 6H, H-10/H-11), 2.04 (s, 3H, H-12), 7.56 (d, $^{3}J_{H-H}$ = 8.17 Hz, 1H, H-7), 7.80 (dd, $^{3}J_{H-H}$ = 8.17 Hz, $^{4}J_{H-H}$ = 1.70 Hz, 1H, H-6), 7.86 (d, $^{4}J_{H-H}$ = 1.70 Hz, 1H, H-4).

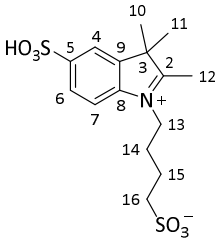
^{13}C {1H} NMR (125.75 MHz, d_6-DMSO)
δ = 15.2 (1C, CH_3, C-12), 22.3 (2C, CH_3, C-10/C-11), 53.2 (1C, C_q, C-2), 118.1 (1C, CH, C-7), 119.2 (1C, CH, C-6), 125.1 (1C, C_q, C-5), 145.2 (1C, CH, C-4), 153.6 (1C, C_q, C-9), 172.8 (1C, C_q, C-8), 188.8 (1C, C_q, C-3).

MS (HR-ESI$^+$)

m/z = 262.0513 $[M+Na]^+$, calculated for $C_{11}H_{13}NNaO_3S^+$: 262.0508
240.0694 $[M+H]^+$, calculated for $C_{11}H_{14}NO_3S^+$: 240.0689.

5-Sulfo-1-(4-sulfobutyl)-2,3,3-trimethyl-*3H*-indolium betaine (IV)

This is a known compound which was synthesized following a procedure by Pham *et al.*[5] (slightly modified).



$C_{15}H_{21}NO_6S_2$
375.46 g/mol

III (1.00 g, 4.18 mmol, 1.0 eq.) and 1,4-butanesultone (1.00 mL, 1.33 g, 9.78 mmol, 2.3 eq.) were suspended in 1,2-dichlorobenzene (25 mL) and stirred at 110 °C for 12 hours under argon. After cooling to room temperature, water was added to the reaction mixture until all solid material was dissolved. The aqueous phase was washed with CH_2Cl_2, the organic layer was discarded. The aqueous layer was evaporated to dryness to yield **IV** as red solid (1.04 g, 2.80 mmol, 66%).

^{1}H NMR (600.13 MHz, D_2O)

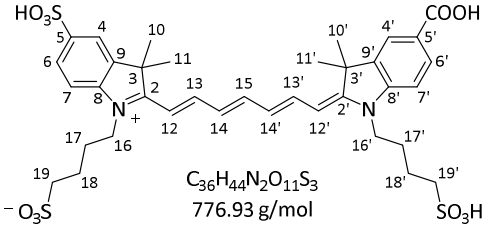
δ = 1.60 (s, 6H, H-10/11), 1.76 - 1.79 (m, 2H, H-15), 1.85 - 1.94 (m, 2H, H-14), 2.97 (t, 2H, $^3J_{H\text{-}H}$ = 7.45 Hz, H-16), 4.12 (t, $^3J_{H\text{-}H}$ = 6.00 Hz, 2H, H-13), 7.91 (d, $^3J_{H\text{-}H}$ = 8.60 Hz, 1H, H-6), 8.02 (d, $^3J_{H\text{-}H}$ = 8.60 Hz, 1H, H-7), 8.11 (s, 1H, H-4); the signal for H-12 was not observed.

MS (HR-ESI$^-$)

m/z = 374.0719 $[M\text{-}H]^-$, calculated for $C_{15}H_{20}NO_6S_2^-$: 374.0738.

Bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5'-carboxylic acid-5-sulfonic acid (1)

This is a known compound which was synthesized following a procedure by Weissleder *et al.*[6] (slightly modified).



$C_{36}H_{44}N_2O_{11}S_3$
776.93 g/mol

IV (200 mg, 0.54 mmol, 1.0 eq.), glutaconaldehyde dianyl monohydrochloride (177 mg, 0.62 mmol, 1.2 eq.) and sodium acetate (146 mg, 1.78 mmol, 3.3 eq.) were dissolved in acetic anhydride (5 mL) and glacial acetic acid (2.5 mL) under argon atmosphere. The mixture was heated to 120 °C and stirred for 45 minutes. Next, **II** (184 mg, 0.54 mmol, 1.0 eq.) dissolved in acetic anhydride (2 mL) and glacial acetic acid (1 mL) was added quickly and heating was continued for 60 minutes. After cooling to room temperature, the solution was poured into Et_2O (300 mL). The green precipitate was collected by filtration and recrystallized from 2-propanol/water (4/1, 11 mL) at 4 °C. The solid material was separated and dried *in vacuo.* Finally, the crude product was dissolved in H_2O with 0.1% TFA, filtered through a pad of C18 silica, and subsequently purified by preparative RP HPLC (Latek Nucleosil C18 (5 μm, 250 mm x 20 mm), H_2O with 0.1% TFA/MeCN, 15 mL/min, 0 min - 0% MeCN, 5 min - 10% MeCN, 30 min - 35% MeCN, t_R = 23.4 min) to obtain **1** as green amorphous powder (47.4 mg, 6.10 μmol, 11%).

^{1}H NMR (600.13 MHz, d_6-DMSO)

δ = 1.62 (s, 6H, H-10/H-11), 1.66 (s, 6H, H10'/H-11'), 1.70 - 1.84 (m, 8H, H-17/H-17'/ H-18/H-18'), 2.56 - 2.64 (m, 4H, H-19/H-19'), 3.93 - 4.06 (m, 2H, H-16), 4.15 - 4.25 (m, 2H, H-16'), 6.27 (d, $^3J_{H-H}$ = 13.29 Hz, 1H, H-12), 6.56 (t, $^3J_{H-H}$ = 12.09 Hz, 1H, H-14), 6.63 (t, $^3J_{H-H}$ = 12.09, 1H, H-14'), 6.70 (d, $^3J_{H-H}$ = 14.04 Hz, 1H, H-12'), 7.32 (d, $^3J_{H-H}$ = 8.25 Hz, 1H, H-7'), 7.50 (d, $^3J_{H-H}$ = 8.25 Hz, 1H, H-7), 7.67 (dd, $^3J_{H-H}$ = 8.25 Hz, $^4J_{H-H}$ = 1.13 Hz, 1H, H-6), 7.23 - 7.79 (m, 2H, H-13/H-15), 7.82 (s, 1H, H-4), 7.91 (dd, $^3J_{H-H}$ = 8.25 Hz, $^4J_{H-H}$ = 1.13 Hz, 1H, H-6'), 7.99 (s, 1H, H-4'), 7.99 - 8.05 (m, 1H, H-13').

MS (HR-ESI$^-$)

m/z = 387.0978 $[M-2H]^{2-}$, calculated for $C_{36}H_{42}N_2O_{11}S_2^{2-}$: 374.0981
257.7291 $[M-3H]^{3-}$, calculated for $C_{36}H_{41}N_2O_{11}S_2^{3-}$: 257.7296.

UV/Vis (PBS)

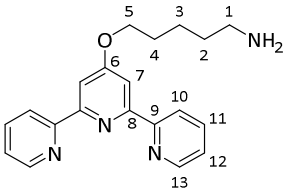
$\lambda_{max\,(Abs)}$ [nm] (ε[$M^{-1}cm^{-1}$]) = 750 nm (270 000).

Fluorescence (PBS)

λ_{Ex} [nm] = 705; $\lambda_{max\,(Em)}$ [nm] = 775; ϕ = 0.1.

5-([2,2':6',2''-terpyridine]-4'-yloxy)pentan-1-amine (V)

This is a known compound which was synthesized following a procedure by Meier and Schubert.[7]



$C_{20}H_{22}N_4O$
334.41 g/mol

Powdered KOH (420 mg, 7.50 mmol, 4.0 eq.) was suspended in anhydrous DMSO (56 mL) at 60 °C and 5-amino-pentan-1-ol (193 mg, 1.87 mmol, 1.0 eq.) was added. The reaction mixture was stirred for 30 minutes. 4'-chloro-2,2':6',2''-terpyridine (501 mg, 1.87 mmol, 1.0 eq.) was added and stirring was continued for 4 hours. Subsequently, the mixture was cooled to room temperature and the resulting orange solution was added dropwise to 950 mL ice-cold deionized water, stirred for 2 hours, filtered, and dried *in vacuo* to yield **V** as white shiny solid (398 mg, 1.19 mmol, 64%).

^{1}H NMR (600.13 MHz, $CDCl_3$)

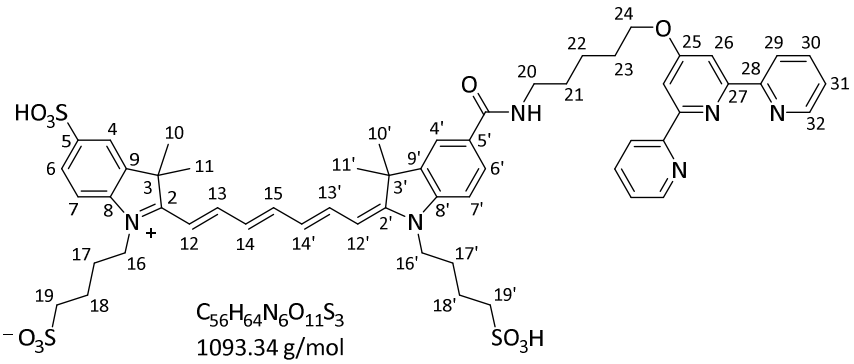
δ = 1.48 - 1.55 (m, 4H, H-2/H-3), 1.85 (qn, $^3J_{H-H}$ = 6.62 Hz, 2H, H-4), 2.70 (m, 2H, H-1), 4.20 (t, $^3J_{H-H}$ = 6.62 Hz, 2H, H-5), 7.33 (m, 2H, H-12), 7.81 (dt, $^3J_{H-H}$ = 7.80 Hz, $^4J_{H-H}$ = 1.75 Hz, 2H, H-11), 7.98 (s, 2H, H-7), 8.59 (m, 2H, H-10), 8.66 (m, 2H, H-13).

^{13}C {^{1}H} NMR (150.92 MHz, $CDCl_3$)

δ = 23.3 (1C, CH_2, C-4), 28.8 (1C, CH_2, C-5), 33.5 (1C, CH_2, C-3), 42.1 (1C, CH_2, C-2), 68.0 (1C, CH_2, C-6), 107.3 (2C, CH, C-8), 121.3 (2C, CH, C-11), 123.7 (2C, CH, C-13), 136.7 (2C, CH, C-12), 148.9 (2C, CH, C-14), 156.1 (2C, C_q, C-10), 157.0 (2C, C_q, C-9), 167.2 (1C, C_q, C-7).

MS (HR-ESI$^+$)
m/z = 335.1868 $[M+H]^+$, calculated for $C_{20}H_{23}N_4O^+$: 335.1866.

Sodium 2-((1*E*,3*E*,5*E*,7*Z*)-7-(5-((5-([2,2':6',2''-terpyridin]-4'-yloxy)pentyl)carbamoyl)-3,3-dimethyl-1-(4-sulfonatobutyl)indolin-2-ylidene)hepta-1,3,5-trien-1-yl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indol-1-ium-5-sulfonate (NIR-Z)



1 (17.1 mg, 22.0 μmol, 1.0 eq.) was dissolved in anhydrous DMF (2 mL) under argon. TBTU (8.48 mg, 26.4 μmol, 1.2 eq.) and DIPEA (10.5 μl, 7.99 mg, 66.0 μmol, 3.0 eq.) were added as solution in anhydrous DMF (1 mL). The mixture was stirred for 30 minutes at 0 °C. Subsequently, 5-([2,2':6',2''-terpyridine]-4'-yloxy)pentan-1-amine (**V**) (16.4 mg, 49.1 μmol, 2.2 eq.) was added. After stirring for three hours at room temperature, the reaction mixture was poured into Et_2O (100 mL). The precipitate was collected by filtration. The solid material was dissolved in H_2O with 0.1% TFA, filtered through a pad of C18 silica, and subsequently purified by semi-preparative RP HPLC (Macherey-Nagel C18 (5 μm, 250 mm x 10 mm), H_2O with 0.1% TFA/MeCN, 4 mL/min, 0 min - 0% MeCN, 1 min - 20% MeCN, 31 min - 50% MeCN, t_R = 21.2 min). **NIR-Z** was obtained as green amorphous powder (14.7 mg, 13.4 μmol, 61%).

^{1}H NMR (600.13 MHz, d_6-DMSO)
δ = 1.51 - 1.53 (m, 2H, H-22), 1.59 (s, 6H, H-10 or H-10'/H-11 or H-11'), 1.63 (s, 6H, H-10 or H-10'/H-11 or H-11'), 1.63 - 1.68 (m, 2H, H-21), 1.68 - 1.83 (m, 8H, H-17/H-17'/H-18/H-18'), 1.88 (qn, $^3J_{H\text{-}H}$ = 7.24 Hz, 2H, H-23), 2.51 - 2.59 (m, 4H, H-19/H-19'), 3.30 - 3.35 (m, 2H, H-20), 3.96 - 4.02 (m, 2H, H-16 or H-16'), 4.10 - 4.18 (m, 2H, H-16 or H-16'), 4.35 (t, $^3J_{H\text{-}H}$ = 6.40 Hz, 2H, H-24), 6.29 (d, $^3J_{H\text{-}H}$ = 13.96 Hz, 1H, H-12 or H-12'), 6.50 - 6.58 (m, 1H, H-14 or H-14'), 6.58 - 6.66 (m, 2H, H-12 or H-12'/ H-14 or H-14'), 7.28 (d, $^3J_{H\text{-}H}$ = 8.22 Hz, 1H, H-7 or H-7'), 7.44 (d, $^3J_{H\text{-}H}$ = 8.22 Hz, 1H, H-7 or H-7'), 7.65 (d, $^3J_{H\text{-}H}$ = 8.22 Hz, 1H, H-6 or H-6'), 7.71 - 7.83 (m, 6H, H-4 or H-4'/H-6 or H-6'/H-13 or H-13'/H-15/H-31), 7.93 (s, 1H, H-4 or H-4'), 7.92 - 7.99 (m, 1H, H-13 or H-13'), 8.14 (s, 2H, H-26), 8.31 (t, $^3J_{H\text{-}H}$ = 7.24 Hz, 2H, H-30), 8.40 - 8.45 (m, 1H, NH), 8.84 - 8.89 (m, 4H, H-29/H-32).

^{13}C {^{1}H} NMR (150.92 MHz, d_6-DMSO)
δ = 22.4 (1C, CH_2, C-17 or C-17' or C-18 or C-18'), 22.4 (1C, CH_2, C-17 or C-17' or C-18 or C-18'), 22.9 (1C, CH_2, C-22), 25.9 (1C, CH_2, C-17 or C-17' or C-18 or C-18'), 26.2 (1C, CH_2, C-17 or C-17' or C-18 or C-18'), 26.9 (2C, CH_3, C-10/C-11 or C-10'/C-11'), 27.4 (2C, CH_3, C-10/C-11 or C-10'/C-11'), 28.1 (1C, CH_2, C-23), 28.8 (1C, CH_2, C-21), 39.0 (1C, CH_2, C-20), 43.1 (1C, CH_2, C-16 or C-16'), 44.1 (1C, CH_2, C-16 or C-16'), 47.8 (1C, C_q), 49.3 (1C, C_q), 50.6 (1C, CH_2, C-19 or C-19'), 50.8 (1C, CH_2, C-19 or C-19'), 68.8 (1C, CH_2, C-24), 103.1* (1C, CH, C-12 or C-12'), 105.8* (1C, CH, C-14 or C-14'), 109.1 (2C, CH, C-26), 109.6 (1C, CH,

C-7 or C-7'), 111.1 (1C, CH, C-7 or C-7'), 114.2 (1C, C_q), 116.1 (1C, C_q), 119.8 (1C, CH, C-4 or C-4'), 121.1 (1C, CH, C-4 or C-4'), 122.9 (2C, CH, C-29 or C-32), 125.7* (1C, CH, C-12 or C-12' or C-14 or C-14'), 125.8* (1C, CH, C-12 or C-12' or C-14 or C-14'), 126.2 (2C, CH, C-6/C-6'), 128.0 (2C, CH, C-31), 129.6 (1C, C_q), 140.3 (1C, C_q), 140.9 (1C, C_q), 141.3 (2C, CH, C-30), 141.8 (1C, C_q), 144.8 (1C, C_q), 145.9 (1C, C_q), 146.9 (2C, CH, C-29 or C-32), 149.5 (1C, CH, C-13 or C-13'), 150.8 (1C, C_q), 152.9 (1C, CH, C-13 or C-13'), 153.0 (1C, C_q), 156.3* (1C, CH, C-15), 158.2 (1C, C_q), 158.5 (1C, C_q), 165.6 (1C, C_q), 167.4 (1C, C_q).
* Not observable in 1D spectra. Signals were detected via indirect excitation (HSQC).

MS (HR-ESI$^-$)
m/z = 545.1822 $[M]^{2-}$, calculated for $C_{56}H_{62}N_6O_{11}S_3^{2-}$: 545.1825.

UV/Vis (Deion. H_2O)
$\lambda_{max\,(Abs)}$ [nm] (ε [($l \cdot mol^{-1} \cdot cm^{-1}$)]) = 753 (263 000).

Fluorescence (Deion. H_2O)
λ_{Ex} [nm] = 705; $\lambda_{max\,(Em)}$ [nm] = 781; ϕ = 0.1.

UV/Vis (PBS)
$\lambda_{max\,(Abs)}$ [nm] (ε [($l \cdot mol^{-1} \cdot cm^{-1}$)]) = 753 (253 000).

Fluorescence (PBS)
λ_{Ex} [nm] = 705; $\lambda_{max\,(Em)}$ [nm] = 781; ϕ = 0.09.

Analytical HPLC
Latek ProSep C18 (5 μm, 250 mm x 4 mm), H_2O with 0.1% TFA/MeCN, 1 mL/min, 0 min - 0% MeCN, 1 min - 20% MeCN, 31 min - 50% MeCN, t_R = 19.9 min.

Cell Experiments

Cell culture

For cell experiments, the following cell lines were used:

Human cervix carcinoma cells (HeLa acc57) were obtained from the DSMZ and cultivated in EMEM (Lonza) with 10% FCS (Biochrom), 1% penicillin/streptomycin (Biochrom) and 1% L-glutamine (Gibco).

Human lung adenocarcinoma cell lines (H1650 and H1975) were obtained from the German Cancer Research Center (DKFZ, Heidelberg) and cultivated in DMEM (Lonza) supplemented with 10% FCS (Biochrom), 1% penicillin/streptomycin (Biochrom), 1% L-glutamine (Gibco).

Human retinal pigment epithelial cell line (hTERT RPE-1) was a kind gift from B. Cerikan (ZMBH, Heidelberg). The cells were cultivated in DMEM F-12 (Gibco) supplemented with 10% FCS (Biochrom), 1% penicillin/streptomycin (Biochrom).

All cell lines were cultivated in a sterile incubator at 37 °C with 95% humidity and 5% CO_2.

Flow Cytometry

Flow cytometry experiments were performed on a C6 cytometer (Accuri). Cells were seeded in 24-well plates 24 h before the measurement. Before treatment, cells were washed with PBS (Gibco) and subsequently treated in triplicates with the given concentrations of **NIR-Z** or **1** in PBS, in the absence or presence of 1, 10 or 100 eq. of $ZnCl_2$ or $FeCl_2$ for 5 minutes or 50 minutes at 37°C. Following incubation, cells were washed with PBS, detached from the well-plate using a cell scraper and fluorescence intensity was measured. All flow cytometry measurements include 10.000 events in the gated area. Fluorescence excitation was at 640 nm, emission was detected using a 780/60 nm filter. The mean fluorescence intensity of this channel was plotted.

Confocal Microscopy

Cells were seeded in 8-well glass slides (ibidi GmbH) (20.000 cells per well) 24 h before the measurement. For staining, the cells were incubated with **NIR-Z** (10 μM) in PBS or PBS supplemented with different concentrations of $ZnCl_2$ or $FeCl_2$ for for 5-7 minutes at 37 °C. Subsequently, the cells were washed with PBS twice and images were taken on a Leica TCS SP5 X confocal microscope.

Images were recorded at 100 Hz or 10 Hz and a resolution of 512x512 pixels. Excitation: pulsed white-light laser (80 MHz, SuperK Extreme, Koheras) at 670 nm, intensity 70 %; detection: photomultipliers, 680 - 800 nm.

Image Analysis

Confocal microscopy images were extracted using Leica LAS AF Lite software. The intensities were measured with ImageJ.

Statistical Analysis

Two-tailed student's t-test was used for statistical analysis. The required level of significance was defined to be 5 % ($p \leq 0.05$).

Spectra

Analytical Data for NIR-Z

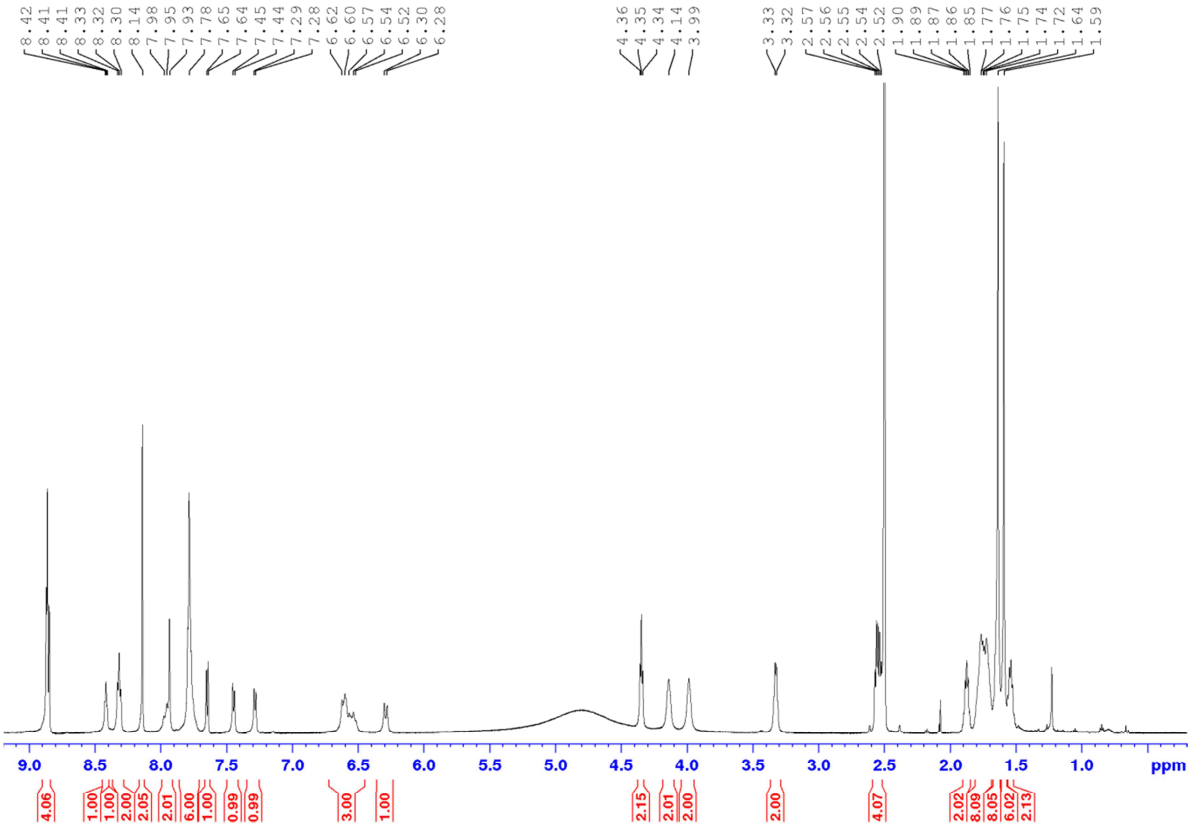


Fig. S12 - ^{1}H NMR of **NIR-Z**, 600.13 MHz, d_6-DMSO.

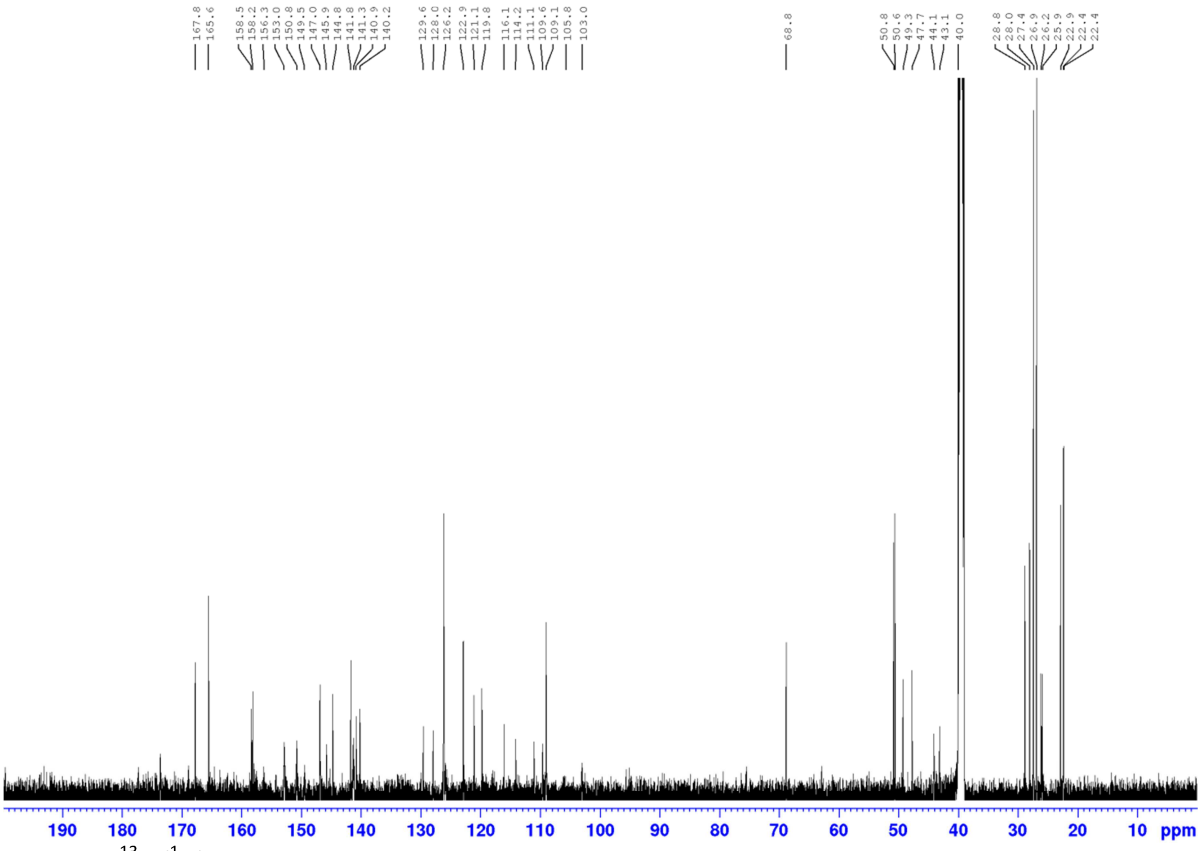


Fig. S13 - ^{13}C {^{1}H} NMR of **NIR-Z**, 150.92 MHz, d_6-DMSO.

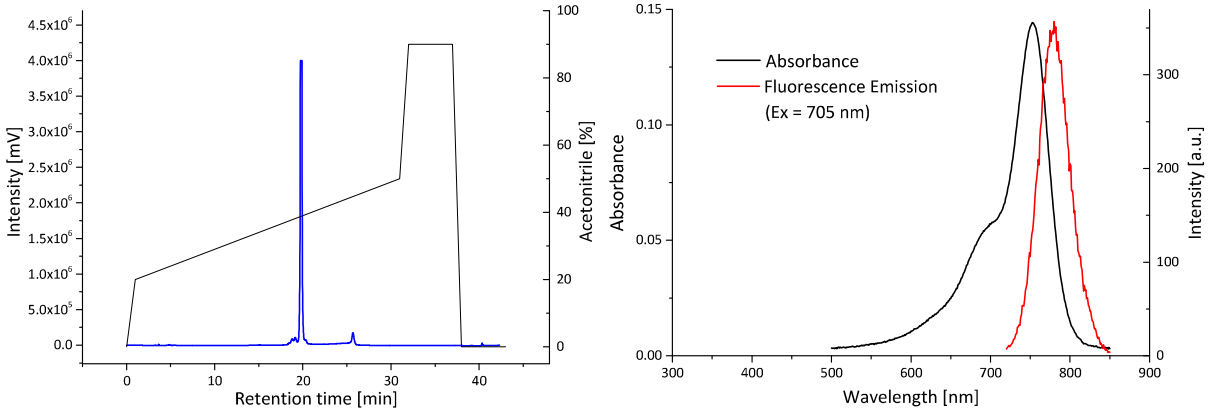


Fig. S14 - **Left:** Analytical HPLC of **NIR-Z** (Latek ProSep C18 (5 μm, 250 mm x 4 mm), deionized H_2O with 0.1% TFA/MeCN, 1 mL/min, 0 min - 0% MeCN, 1 min - 20% MeCN, 31 min - 50% MeCN; t_R = 19.9 min). **Right:** UV/Vis absorbance and fluorescence emission spectra of **NIR-Z** (0.56 μM solution in deionized H_2O; $\lambda_{max,Abs}$ = 753 nm, $\lambda_{max,Em}$ = 781 nm).

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