Supporting Information

Supramolecular Conjugated Polymer Materials for Organelle Imaging in Living Cells

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Experimental Section

Materials and Measurements: All chemicals were purchased from Sigma-Aldrich Chemical Company, or Beijing Chemical Works and used as received. PT was synthesized according to the procedure in literatures.[1] HeLa cells were obtained from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Science (Beijing, China). Fetal bovine serum (FBS) was purchased from Sijiqing Biological Engineering Materials. PBS, DMEM (Dulbecco's modified Eagle medium) and Opti-MEM reduced serum medium were purchased from HyClone/Termo Fisher Scientific (Beijing, China). Water was purified with a Millipore filtration system. MTT was purchased from Tiangen Biotech (Beijing, China). Lipo 2000, LysoTracker, MitoTracker, GolgiTracker and ERTracker were purchased from Life Technologies (Beijing, China). Tat-His_6 peptide was purchased from SBS Genetech Co. Ltd. (Beijing, China). RFP-His_{10} protein was purchased from Yingrun Biotechnology Co., Ltd (Changsha, China). pCDNA3.1-moRED-GolgiH10, pCDNA3.1-moRED-MitoH10 and pCDNA3.1-moRED-ERH10 plasmids were constructed by Yingrun Biotechnology Co., Ltd (Changsha, China). Confocal laser scanning microscope (CLSM) characterization was conducted with a confocal laser scanning biological microscope (FV1000-IX81, Olympus, Japan). The absorbance for MTT analysis was recorded on a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 570 nm. Ultrafiltration tubes were purchased from Minipore (50 kd, USA). Automated cell counter (Countess, Invitrogen) was employed for cell

counting. Zeta potentials and size were carried out on a Nano ZS (ZEN3600, U.K.) system. Agarose gel electrophoresis was conducted on electrophoresis apparatus (DYY-6C, Beijing, China). Calorimetric measurements were carried out on a TAM III microcalorimetric system ((Thermometric AB, Sweden). X-ray photoelectron spectroscopy was carried on ESCALAB250XI surface analysis system (Thermo Scientific, USA).

The preparation of PT/Ni^{2+}/Tat-His_6 complex: 2 μL of PT (10 mM) stock solution was added into 1 mL of H_2O, and then 2 μL of Tat-His_6 (10 mM) and 2 μL of Ni^{2+} ($NiCl_2$, 20 mM) were added into the above solution followed by mixing gently. The prepared complex solution was purified by using ultrafiltration tubes with the molecular cutoff of 50 kd and washed by H_2O for three times. The concentrated solution was dispersed in PBS (2×) to afford final PT/Ni^{2+}/Tat-His_6 complex solution. The concentration of complex was quantified as that of PT (10 μM in RUs).

PT/Ni^{2+}/Tat-His_6 complex for organelle imaging: (a) HeLa cells were cultured at 30 mm cell culture dish at 37 °C overnight. After reaching 80% confluence, HeLa cells were cultured with 2 mL of Opti-MEM reduced serum medium. 4 μg of plasmid (pCDNA3.1-moRED-GolgiH10, pCDNA3.1-moRED-MitoH10 and pCDNA3.1-moRED-ERH10) and 10 μL of Lipo 2000 were mixed at Opti-MEM reduced serum medium and added to cell culture dish gently. After incubation for 4 h, the opti medium was changed into 2 mL of DMEM with 10% FBS. After 72 h, cells were ready for next use. (b) The transfected HeLa cells were seeded at a 6-well culture chamber slide, and cells were washed by PBS for 3 times and exposed to 1 mL of chloroquine (0.25 mM) in PBS for 30 min. After washed by PBS, HeLa cells were incubated with 1 mL of PT/Ni^{2+}/Tat-His_6 complex (10 μM) in PBS for 1 h at 37 °C followed by 3 h incubation in 1 mL of DMEM with 10% FBS. The specimens were examined by CLSM with a 488 nm laser for PT and 559 nm for red fluorescence protein.

Isothermal Titration Microcalorimetry (ITC): Calorimetric measurements were carried out at 25.00 ± 0.01 °C on a TAM III microcalorimetric system with a stainless-steel sample cell of 1 mL. All measurements were operated in PBS buffer. 1) The cell was initially loaded with 0.6 mL Tat-His_6 (10 μM and 200 μM), RFP-His_{10} (10 μM), PT (200 μM) or PT/Tat-His_6 (10 μM) solutions, respectively. And then Ni^{2+} solutions (4-fold to the concentration of component) were injected consecutively into the stirred sample cell in portions of 10 μL via a 500 μL Hamilton syringe controlled by a 612 Thermometric Lund pump until the desired concentration range had been covered, respectively. The observed enthalpy values (ΔH_{obs}) were obtained from the areas of the calorimetric peaks after titrations. The reference cell was filled with 765 μL buffer solutions. The final dilution enthalpies of Ni^{2+} were subtracted from the corresponding observed enthalpy curve of the Ni^{2+}/PT, Ni^{2+}/ RFP-His_{10}, Ni^{2+}/Tat-His_6 or Ni^{2+}/PT+Tat-His_6 titrating curves. Thus the final ΔH_{obs} curves reflect the interactions of Ni^{2+} with polymer or protein. Each ITC curve was repeated at least twice with a deviation less than ± 4%.

2) The cell was initially loaded with 0.6 mL RFP-His_{10} (10 μM) or Tat-His_6 (10 μM) PBS solution, and then the mixture of 40 μM PT-Ni^{2+} PBS solutions were injected consecutively into the stirred sample cell in portions of 10 μL via a 500 μL Hamilton syringe controlled by a 612 Thermometric Lund pump until the desired concentration range had been covered. The observed enthalpy values (ΔH_{obs}) were obtained from the areas of the calorimetric peaks after titrations. The reference cell was filled with 765 μL buffer solutions. The final dilution enthalpies of Ni^{2+} were subtracted from the corresponding observed enthalpy curves of titrating PT-Ni^{2+} mixtures into RFP-His_{10} or Tat-His_6 solutions. Thus the final ΔH_{obs} curves reflect the interactions of PT-Ni^{2+} with proteins. Each ITC curve was repeated at least twice with a deviation less than ± 4%.

X-ray photoelectron spectroscopy (XPS):5 μL of PT/Ni^{2+}/Tat-His_6 complex (100 μM), Tat-His_6 (100 μM) and PT (100 μM) were added to silicon pellet followed by vacuum drying for 5 h under 45°C. Then Multi-function energy spectrum analysis was carried.

Size and zeta potential assay of PT and PT/Ni^{2+}/Tat-His_6 complex: (1) For PT and PT/Ni^{2+}/Tat-His_6 complex: 1 μL of PT (10 mM) was dissolved in 1 mL of H_2O, 1μL of PT and 1 μL of Ni^{2+} were dissolved in 1 mL of H_2O, 1μL of PT and 1 μL of Tat-His_6 were dissolved in 1 mL of H_2O or 1 mL of PT/Ni^{2+}/Tat-His_6 (10 μM) complex in H_2O followed by measurement of size and zeta potential on Nano ZS system. (2) For PT/Ni^{2+}/Tat-His_6 complex incubated with RFP-His_{10}: after preparing complex, 20 μg of RFP-His_{10} was added to 1 mL of PT/Ni^{2+}/Tat-His_6 complex (10 μM) solution and incubated for 30 min at 37 °C followed with size and zeta potential measurements.

Cell culture: HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% carbon dioxide at 37 °C.

Cell viability assay: HeLa cells were seeded in 96-well plate with 8×10^3 cells per well. After incubating overnight, cells were incubated with PT1/Ni^{2+}/Tat-His_6, PT/Ni^{2+}/Tat-His_6 and PT3/Ni^{2+}/Tat-His_6 complex respectively in DMEM (10% FBS) with the final concentration ranging from 0 μM to 50 μM (100 μL /well). 24 h later, 100 μL of MTT (10 μM) in DMEM (10% FBS) was added, and 4 h incubation was needed. After removing the culture medium, 100 μL of DMSO was added to every well. The absorption was recorded by a microplate reader at 570 nm after shaking for 2 min. The cell viability ratio (VR) was evaluated according to the following equation:

$$VR = \frac{A}{A_0} \times 100\%$$

Where A_0 is the absorbance of cells without any drugs, and A is the absorbance of cells incubated with complex.

Cellular uptake of PT/Ni^{2+}/Tat-His_6 complex: For PT/Ni^{2+}/Tat-His_6 complex: HeLa cells were seeded at a 6-well culture chamber slide, and cells were washed by PBS for 3 times and exposed to 1 mL of chloroquine (0.25 mM) in PBS for 30 min. After washed by PBS, HeLa cells were incubated with 1 mL of PT/Ni^{2+}/Tat-His_6 complex (10 μM) in PBS for 1 h at 37 °C followed by 3 h incubation in 1 mL of DMEM with 10% FBS. The specimens were examined by CLSM with a 488 nm laser. As control, the incubation conditions of PT/Ni^{2+}, PT/Tat-His_6 and PT were as same as PT/Ni^{2+}/Tat-His_6 complex, and the final concentrations of these complexes were 10 μM.

The measurement of internalization speed for PT/ Ni^{2+}/Tat-His_6: HeLa cells were seeded at a 30 mm culture chamber slide, and cells were washed by 1 mL of PBS for 3 times and exposed to 1 mL of chloroquine (0.25 mM) in PBS for 30 min. After washed by PBS for three times, HeLa cells were incubated with 1 mL of PT/Ni^{2+}/Tat-His_6 complex (10 μM) in PBS for 1 h at 37 °C. Then cells were incubated in DMEM with 10% FBS for 0 h, 1 h and 3 h respectively, followed by incubation with 1 mL of DiD (2 μM) for 30 min and 1 mL of Hoechst 33342 (1 μM) in PBS for 10 min under dark at 37 °C. The specimens were examined by CLSM with a 405 nm laser for Hoechst, a 488 nm laser for PT and a 635 nm laser for DiD.

The stabilization of PT/Ni^{2+}/RFP-His_{10} complex inside transfected HeLa cells: After incubated with 1 mL of PT/Ni^{2+}/Tat-His_6 complex (10 μM) in PBS for 1 h, two groups of transfected HeLa cells were cultured in 1 mL of DMEM with 10% FBS for 3 h and 23 h,

respectively. The specimens were examined by CLSM with a 488 nm laser for PT and 559 nm for RFP.

Colocalization of PT/Ni^{2+}/Tat-His_6 complex with organelle dyes (ERTracker Red, MitoTracker Red, LysoTracker Red and GolgiTracker): HeLa cells were exposed to 1 mL of chloroquine (0.25 mM) in PBS for 30 min and incubated with 1 mL of PT/Ni^{2+}/Tat-His_6 complex (10 μM) as described above. After a brief PBS wash, cells were cultured with 1mL of ERTracker Red, MitoTracker Red, and LysoTracker Red in PBS with the final concentration of 1 μM under dark for 30 min respectively. For GolgiTracker, cells were cultured with it in 1 mL of PBS with the final concentration 1 μM under 4 °C for 30 min followed by incubation in DMEM with 10% FBS at 37 °C for 30 min sequentially. Cells were washed by PBS for three times and then observed by CLSM. PT was excited by 488 nm laser and that of organelle dyes were 559 nm laser.

Plasmids: The sequence of plasmids encoding for Golgi-RFP-His_{10}, ER-RFP-His_{10} and Mito-RFP-His_{10} are listed as follows:

(1) pCDNA3.1- moRED-GolgiH10 (expression of Golgi-RFP-His_{10}):

ATGAGGCTTCGGGAGCCGCTCCTGAGCGGCAGCGCCGCGATGCCAGGCGCGTCC
CTACAGCGGGCCTGCCGCCTGCTCGTGGCCGTCTGCGCTCTGCACCTTGGCGTCA
CCCTCGTTTACTACCTGGCTGGCCGCGACCTGAGCCGCCTGCCCCAACTGGTCGG
AGTCTCCACACCGCTGCAGGGCGGCTCGAACAGTGCCGCCGCCATCGGGCAGTCC
TCCGGGGAGCTCCGGACCGGAGGGGCCAAGGATCCACCGGTCGCCACCATGGAC
AACACCGAGGACGTCATCAAGGAGTTCATGCAGTTCAAGGTGCGCATGGAGGGC
TCCGTGAACGGCCACTACTTCGAGATCGAGGGCGAGGGCGAGGGCAAGCCCTAC
GAGGGCACCCAGACCGCCAAGCTGCAGGTGACCAAGGGCGGCCCCCTGCCCTTC
GCCTGGGACATCCTGTCCCCCCAGTTCCAGTACGGCTCCAAGGCCTACGTGAAGC

ACCCCGCCGACATCCCCGACTACATGAAGCTGTCCTTCCCCGAGGGCTTCACCTG
GGAGCGCTCCATGAACTTCGAGGACGGCGGCGTGGTGGAGGTGCAGCAGGACTC
CTCCCTGCAGGACGGCACCTTCATCTACAAGGTGAAGTTCAAGGGCGTGAACTTC
CCCGCCGACGGCCCCGTAATGCAGAAGAAGACTGCCGGCTGGGAGCCCTCCACC
GAGAAGCTGTACCCCCAGGACGGCGTGCTGAAGGGCGAGATCTCCCACGCCCTG
AAGCTGAAGGACGGCGGCCACTACACCTGCGACTTCAAGACCGTGTACAAGGCC
AAGAAGCCCGTGCAGCTGCCCGGCAACCACTACGTGGACTCCAAGCTGGACATC
ACCAACCACAACGAGGACTACACCGTGGTGGAGCAGTACGAGCACGCCGAGGCC
CGCCACTCCGGCTCCCAGGGACATCACCACCATCATCACCATCACCACCACTAG

(2) pCDNA3.1-moRED-MitoH10 (expression of Mito-RFP-His_{10}):

ATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTTGACAGGCTCGGCCCGGCGGC
TCCCAGTGCCGCGCGCCAAGATCCATTCGTTGGGGGATCCACCGGTCGCCACCAT
GGACAACACCGAGGACGTCATCAAGGAGTTCATGCAGTTCAAGGTGCGCATGGA
GGGCTCCGTGAACGGCCACTACTTCGAGATCGAGGGCGAGGGCGAGGGCAAGCC
CTACGAGGGCACCCAGACCGCCAAGCTGCAGGTGACCAAGGGCGGCCCCCTGCC
CTTCGCCTGGGACATCCTGTCCCCCCAGTTCCAGTACGGCTCCAAGGCCTACGTG
AAGCACCCCGCCGACATCCCCGACTACATGAAGCTGTCCTTCCCCGAGGGCTTCA
CCTGGGAGCGCTCCATGAACTTCGAGGACGGCGGCGTGGTGGAGGTGCAGCAGG
ACTCCTCCCTGCAGGACGGCACCTTCATCTACAAGGTGAAGTTCAAGGGCGTGAA
CTTCCCCGCCGACGGCCCCGTAATGCAGAAGAAGACTGCCGGCTGGGAGCCCTCC
ACCGAGAAGCTGTACCCCCAGGACGGCGTGCTGAAGGGCGAGATCTCCCACGCC
CTGAAGCTGAAGGACGGCGGCCACTACACCTGCGACTTCAAGACCGTGTACAAG
GCCAAGAAGCCCGTGCAGCTGCCCGGCAACCACTACGTGGACTCCAAGCTGGAC
ATCACCAACCACAACGAGGACTACACCGTGGTGGAGCAGTACGAGCACGCCGAG
GCCCGCCACTCCGGCTCCCAGGGACATCACCACCATCATCACCATCACCACCACT
AG

(3) pCDNA3.1-moRED-ERH10 (expression of ER-RFP-His_{10}):

ATGCTGCTATCCGTGCCGTTGCTGCTCGGCCTCCTCGGCCTGGCCGTCGCCGGGG
ATCCACCGGTCGCCACCATGGACAACACCGAGGACGTCATCAAGGAGTTCATGC
AGTTCAAGGTGCGCATGGAGGGCTCCGTGAACGGCCACTACTTCGAGATCGAGG
GCGAGGGCGAGGGCAAGCCCTACGAGGGCACCCAGACCGCCAAGCTGCAGGTGA
CCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCCAGTA
CGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACATGAAGCTG
TCCTTCCCCGAGGGCTTCACCTGGGAGCGCTCCATGAACTTCGAGGACGGCGGCG
TGGTGGAGGTGCAGCAGGACTCCTCCCTGCAGGACGGCACCTTCATCTACAAGGT
GAAGTTCAAGGGCGTGAACTTCCCCGCCGACGGCCCCGTAATGCAGAAGAAGAC
TGCCGGCTGGGAGCCCTCCACCGAGAAGCTGTACCCCCAGGACGGCGTGCTGAA
GGGCGAGATCTCCCACGCCCTGAAGCTGAAGGACGGCGGCCACTACACCTGCGA
CTTCAAGACCGTGTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCAACCACTA
CGTGGACTCCAAGCTGGACATCACCAACCACAACGAGGACTACACCGTGGTGGA
GCAGTACGAGCACGCCGAGGCCCGCCACTCCGGCTCCCAGGGAAAGGACGAGCT
GTAATAG

References

[1] C. Xing, Q. Xu, H. Tang, L. Liu, S. Wang, *J. Am. Chem. Soc.* **2009**, *131*, 13117-13124.

	PT/Ni^{2+}	PT/Tat-His_6
Size (nm)	19±4	78±11
Zeta (mV)	-12±1	22±1

Table S1. Size and zeta potential distribution of PT/Ni^{2+} and PT/Tat-His_6.

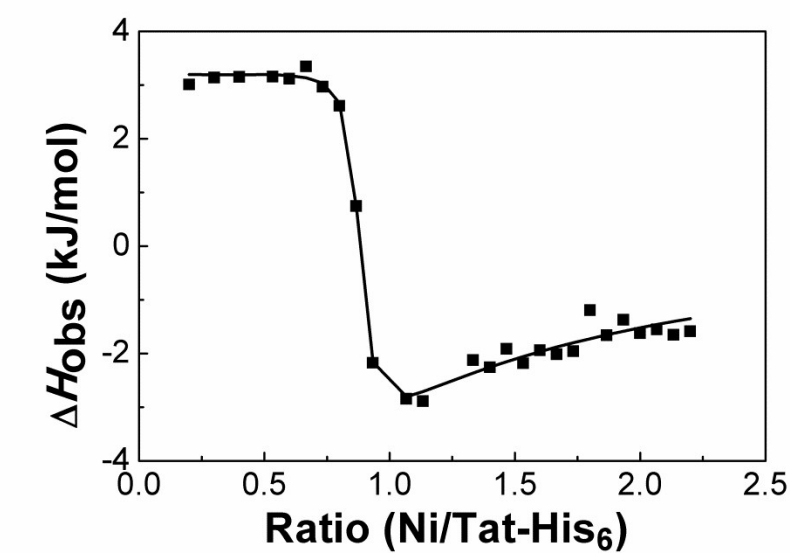

Figure S1. The observed enthalpy changes (ΔH_{obs}) against the molar ratio of Ni^{2+}/Tat-His_6 (R_{Ni}) for titrating Ni^{2+} (800 μM) into Tat-His_6 (200 μM).

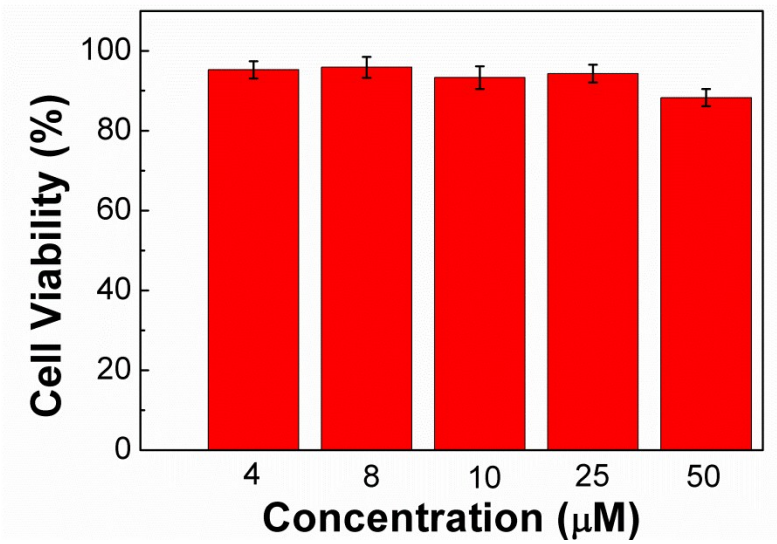

Figure S2. Cell cytotoxicity of PT/Ni^{2+}/Tat-His_6 complex. [PT/Ni^{2+}/Tat-His_6] = 0 ~ 50 μM. The concentration of complex is quantified as that of PT.

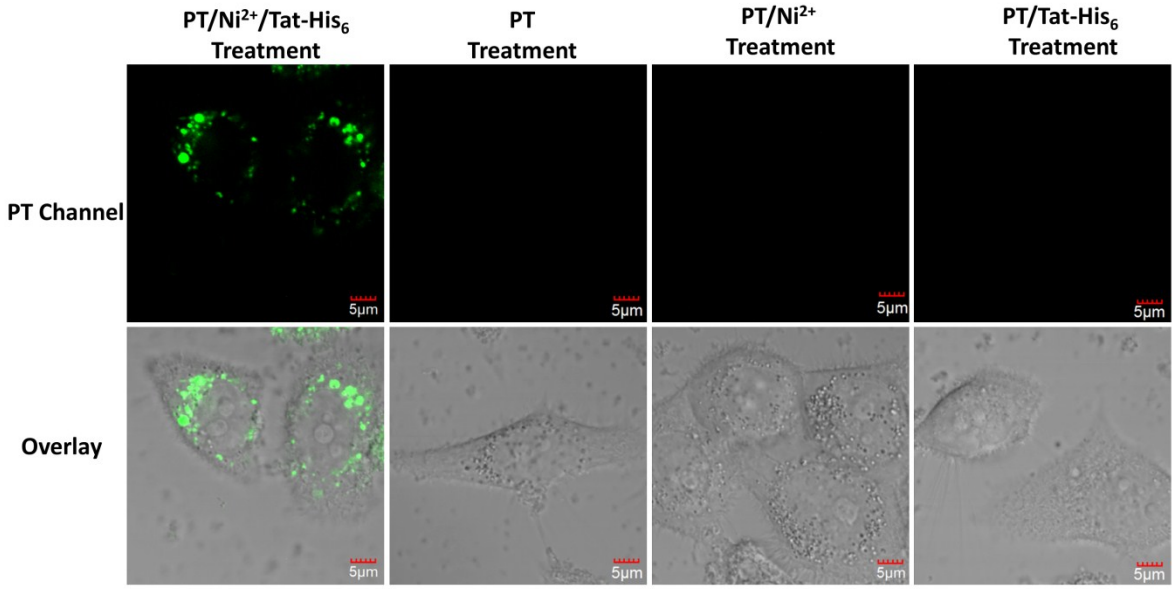

Figure S3. CLSM images of HeLa cells incubated with PT/Ni^{2+}/Tat-His_6, PT, PT/Ni^{2+} and PT/Tat-His_6, respectively. [PT] = [PT/Ni^{2+}] = [PT/Tat-His_6] = [PT/Ni^{2+}/Tat-His_6] = 10 μM. The concentration of complexes are quantified as PT concentration.

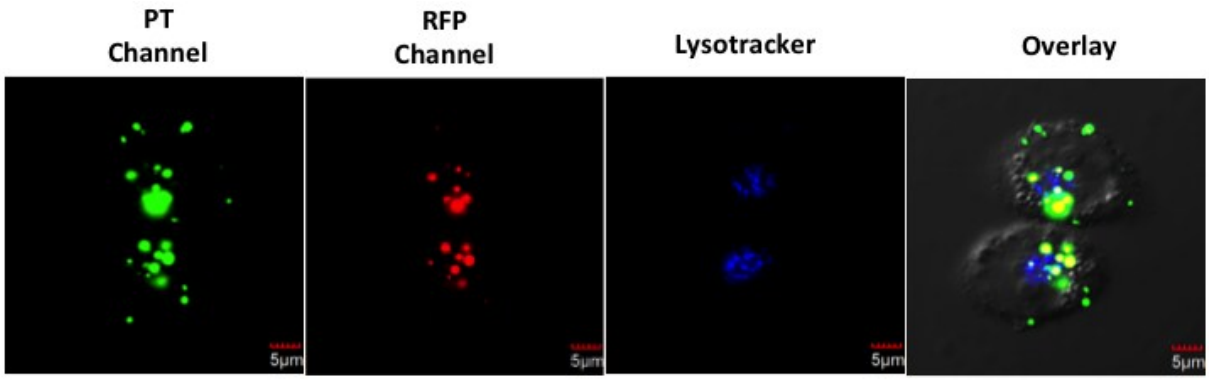

Figure S4. CLSM images of HeLa cells incubated with complex for 4 h after transfected with pCDNA3.1-moRED-GolgiH10 plasmid for 72 h followed by incubation with Lyso Tracker for 30 min. [PT/Ni^{2+}/Tat-His_6] = 10 μM, [LysoTracker] = 1 μM.

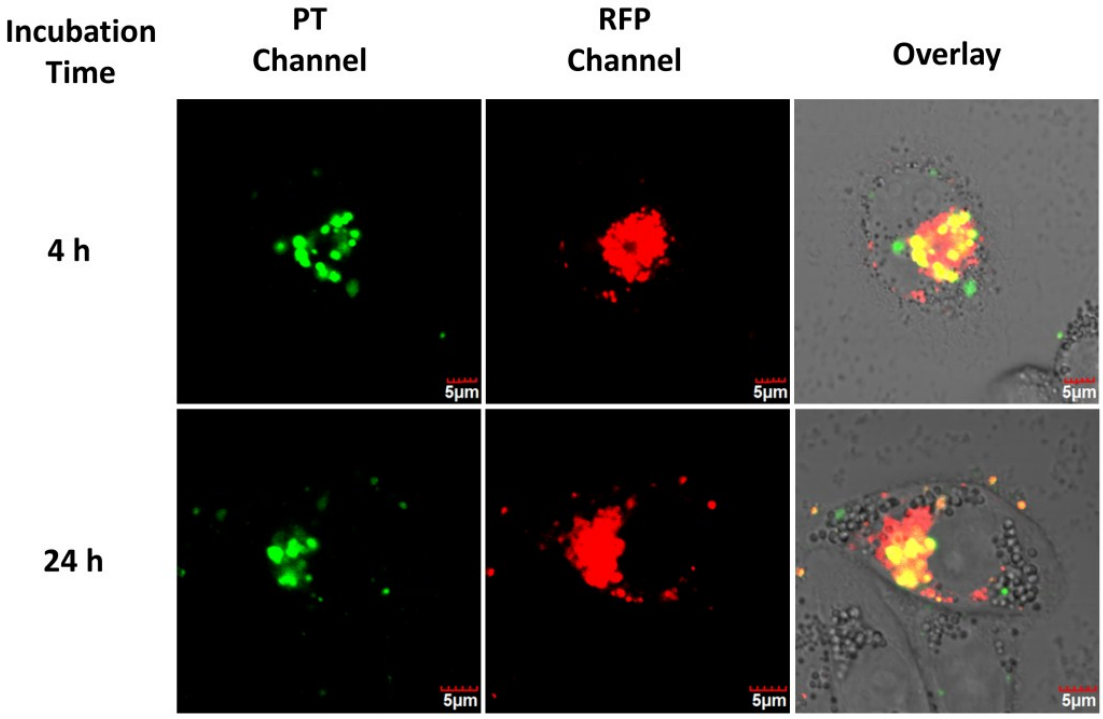

Figure S5. CLSM images of HeLa cells transfected by pCDNA3.1-moRED-GolgiH10 plasmid with the treatment of PT/Ni^{2+}/Tat-His_6 complex. [PT/Ni^{2+}/Tat-His_6] = 10 μM. The false color of PT was green, and the false color of RFP was red.