SUPPORTING INFORMATION

Cellular Delivery of Nanoparticles Revealed with Combined Optical and Isotopic Nanoscopy

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1. Experimental details

Materials
All reagents were purchased from VWR, Alfa Aesar or Sigma-Aldrich and used without further purification. Sealed ampules of DMF-d$_7$ (Cambridge Isotopes) were used without further purification. Modified second generation Grubbs’ ruthenium initiator, ($\text{IMesH}_2$)((CH$_3$N)$_2$Cl)$_2$Ru=CHPh, was prepared as previously described (Sanford et al. Organometallics, 2001, 20, 5314). Polymerizations were performed under a dry N$_2$ atmosphere. HeLa and A549 cells were obtained from ATCC. $^1$H NMR spectra were recorded on a Varian Mercury Plus spectrometer (400 MHz). Polymer dispersities and molecular weights were determined by size-exclusion chromatography (Phenomenex Phenogel 5u 10, 1k-75k, 300 x 7.80 mm in series with a Phenomenex Phenogel 5u 10, 10K-1000K, 300 x 7.80 mm (0.05 M LiBr in DMF)) using a Shimadzu pump equipped with a multi-angle light scattering detector (DAWN-HELIOS: Wyatt Technology) and a refractive index detector Wyatt Optilab TrEX normalized to a 30,000 MW polystyrene standard. Particle diameters were determined by dynamic light scattering (DLS, $D_{\text{DLS}}$) using a Wyatt Dynapro NanoStar and via Nanoparticle Tracking Analysis (NTA, $D_{\text{NTA}}$). Zeta potential was measured using a Zetasizer (3000 HS, Malvern Instruments Ltd, Worcestershire, UK). TEM images were acquired on carbon grids (Ted Pella, INC.) on a FEI Tecnai G2 Sphera at 200 KV. Pt was quantified by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 3000DV spectrometer in the laboratory of Dr. Paterno Castillo at the Scripps Institution of Oceanography, University of California, San Diego. The absorbance at 595 nm for the cytotoxicity assays was measured using a microtiter plate reader (PerkinElmer Health Sciences Inc., EnSpire multimode plate reader basic unit). A Zeiss ELYRA super resolution microscope located within the Environmental Molecular Sciences Laboratory at PNNL in Richland, WA was used for SIM imaging. A NanoSIMS 50L (Cameca, France) located within the Environmental Molecular Sciences Laboratory at PNNL in Richland, WA was used for secondary ion mass spectrometry analysis.

Synthesis
Synthesis of exo-5-norborneneamidomalonic acid diethyl ester
The ligand was synthesized according to the Kaminski reaction (Synthesis 1987, 917) and Caron et al. protocol (ChemMedChem 2009, 4, 1677).
Synthetic route of exo-5-norborneneamidomalonic acid diethyl ester.

In a 50 mL flask with stirring at r.t., exo-5-norbornene carboxylic acid (248 mg, 2.00 mmol) was dissolved in 5 mL DMF. 2,4,6-chlorodimethoxytriazine (CDMT, 353 mg, 2.02 mmol) was added. The reaction mixture was cooled to 0 °C with an ice bath and N-methylmorpholine (220 µl, 2.00 mmol) was added. The mixture reacted at 0 °C for 5 h and then 2-aminomalonic acid diethyl ester hydrochloride (427 mg, 2.02 mmol) and N-methylmorpholine (220 µl, 2.00 mmol) dissolved in 2.5 mL DMF were added. The mixture reacted under stirring for 2 h at 0 °C and then for overnight at r.t. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate. The solution was successively washed with water, 10% citric acid solution, water, saturated NaHCO₃ solution, water, saturated NaCl solution and water. The organic layer was dried over CaSO₄ and the solvent evaporated. The final product was isolated by column chromatography (75% hexanes, 25% ethyl acetate). Yield: 560 mg, 94%. \(^1\)H NMR (CDCl₃-d₅): \(\delta =\) 6.47 (d, 1H, C(O)NH), 6.11 (m, 2H, CH=CH), 5.12 (d, 1H, NHCH), 4.24 (m, 4H, CH₂CH₃), 2.97 (s, 1H, CHCH), 2.89 (s, 1H, CHCH₂), 2.12 (m, 1H, CHC(O)), 1.91 (m, 1H, CH₂CH(O)), 1.62 (m, 1H, CH₂CH), 1.30 (m, 9H, CH₃, CH₂CH(O), CH₂CH) ppm; HR-MS (ESI-TOF): m/z (%) [M+Na]+ calcd for C₁₅H₂₅NO₅: 318.1317, found: 318.1315.

Synthesis of cis-(cyclohexane-1,2-diamine)(exo-5-norborneneamidomalonate-O,O')platinum(II)

i) Deprotection of exo-5-norborneneamidomalonic acid diethyl ester. exo-5-Norborneneamidomalonic acid diethyl ester (35 mg, 0.12 mmol) was reacted with KOH (13 mg, 0.23 mmol) in water (3 ml) to give the corresponding potassium salt.

ii) Synthesis of the Pt(II) complex. cis-[PtCl₂(NH₃)₂] (44 mg, 0.12 mmol) was suspended in water (3 ml) and AgNO₃ (40 mg, 0.23 mmol) was added in one portion. The mixture was stirred at 40 °C overnight in the dark. It was then filtered to remove AgCl and the potassium salt of exo-5-norborneneamidomalonate was added to the filtrate. The mixture was stirred overnight and the Pt(II) complex precipitated. The product was isolated by filtration, was washed with water, ethanol and diethyl ether and finally was dried under vacuum. Yield: 40 mg, 61%. \(^1\)H NMR (DMSO-d₆): \(\delta =\) 7.51 (d, 1H, NH), 6.08 (m, 2H, CH=CH), 5.96 (m, 2H, NH₂), 5.57 (d, 1H, CH), 5.36 (m, 2H, NH₂), 2.78 (m, 2H, CHCH₂), 2.24 (m, 1H), 2.04 (m, 2H), 1.76 (m, 3H), 1.55 (t, 1H), 1.41 (m, 2H), 1.13 (m, 4H), 0.96 (m, 2H) ppm. HR-MS (ESI-TOF): m/z (%) [M+Na]+ calcd for C₁₇H₂₉N₃O₇Pt: 569.1336, found: 569.1335.
Synthesis of \(^{15}\text{N}-\text{benzyl}\)-5-norborene-exo-2,3-dicarboximide

i) Synthesis of \(^{15}\text{N}-5\)-norborene-exo-2,3-dicarboximide was carried out according to Mansfeld et al. (Org. Biomol. Chem. 2009, 7, 4289).

A mixture of cis-5-norborene-exo-2,3-dicarboxylic anhydride (820 mg, 5 mmol) and \(^{15}\text{N}\)-urea (360 mg, 6 mmol) was heated at 145 °C for 4 h. Water (20 mL) was added and the solution was heated until it was homogenous. A white-grey solid precipitated upon cooling. It was filtered, washed with cold water and dried. Yield: 635 mg (78%). \(^1\text{H}\) NMR (CDCl\textsubscript{3} - d\textsubscript{1}): \(\delta = 8.01\) (d, 1H, NH), 6.28 (m, 2H, \(\text{CH}=\text{CH}\)), 3.29 (s, 2H, \(\text{CHCH}\)), 2.73 (s, 2H, \(\text{CH(O)}\)), 1.58 (d, 1H, \(\text{CH}_2\text{CH(O)}\)), 1.45 (d, 1H, \(\text{CH}_2\text{CH(O)}\)) ppm. HR-MS (ESI-TOF): m/z (%) [M-H]\(^+\) calcd for C\textsubscript{9}H\textsubscript{9}\(^{15}\text{N}\)O\textsubscript{2}: 163.0531, found: 163.0531.

ii) Synthesis of \(^{15}\text{N}-\text{benzyl}\)-5-norborene-exo-2,3-dicarboximide

\(^{15}\text{N}\)-5-norborene-exo-2,3-dicarboxyimide (815 mg, 5 mmol), benzyl bromide (1026 mg, 6 mmol) and K\textsubscript{2}CO\textsubscript{3} (759 mg, 5.5 mmol) in 20 mL acetone were refluxed for 45 h. The solvent was removed and the product was washed with water and dried under vacuum. Yield 945 mg (74%). \(^1\text{H}\) NMR (CDCl\textsubscript{3}, d\textsubscript{1}): \(\delta = 7.25\text{-}7.35\) (m, 5H, Ar), 6.24 (s, 2H, \(\text{CH}=\text{CH}\)), 3.21 (s, 2H, \(\text{CH}_2\)), 2.64 (s, 2H, \(\text{CH(O)}\)), 1.37 (d, 1H, \(\text{CH}_2\text{CH(O)}\)), 1.03 (d, 1H, \(\text{CH}_2\text{CH(O)}\)) ppm. \(^{15}\text{N}\) NMR (CDCl\textsubscript{3} - d\textsubscript{1}): \(\delta = 186\) ppm. HR-MS (ESI-TOF): m/z (%) [M+H]\(^+\) calcd for C\textsubscript{16}H\textsubscript{15}\(^{15}\text{N}\)O\textsubscript{2}: 255.1143, found: 255.1146.

Synthesis of Cy5.5 monomer

i) Synthesis of 3-(2-((1E,3Z,5E)-3-(5-carboxypyridin-2-yl)-5-(1,1-dimethyl-3-(3-sulfopropyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)penta-1,3-dien-1-yl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)propane-1-sulfonate

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3-(1,1,2-trimethyl-1H-benzo[e]indol-3-ium-3-yl)propane-1-sulfonate (1.60 g, 4.9 mmol), 6-(1,3-dioxopropan-2-yl)nicotinic acid (0.43 g, 2.2 mmol), and sodium acetate (1.30 g, 16.1 mmol) was dissolved in 125 mL of a 1:1 mixture of acetic anhydride and acetic acid and heated to reflux for 5 h. Once complete, the solvent was removed by rotary evaporation and the blue solid was dissolved in water with 0.5% TFA and purified by reverse phase HPLC using a gradient from of 10-60% buffer B where buffer A is water with 0.1% TFA and buffer B is acetonitrile with 0.1% TFA. The pure fractions were collected and lyophilized. Yield: 1.04 g, 56%. \(^1\text{H}\) NMR (DMSO-d\textsubscript{6}): \(\delta = 9.32\) (s, 1H), 8.74 (d, 1H), 8.70 (d, 2H), 8.25 (d, 2H), 8.07 (t, 4H), 7.90 (d, 1H), 7.83 (d, 2H),
7.69 (t, 2H), 7.53 (t, 2H), 6.04 (b, 2H), 4.32 (t, 4H), 2.53 (t, 4H), 2.00 (m, 4H), 1.99 (s, 12H). HR-MS (ESI-TOF): m/z (%) [M-H]⁻ calcd for C₄₃H₄₅N₃O₈S₂: 818.2648, found: 818.4485.

ii) Synthesis of 3-(2-((1E,3Z,5E)-5-(1,1-dimethyl-3-(3-sulfopropyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-(5-((2,1,3-dioxo-1,3,3a,4,7a-hexahydro-2H-4,7-methanoisoindol-2-yl)ethyl)carbamoyl)pyridin-2-yl)penta-1,3-dien-1-yl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)propane-1-sulfonate

Cy5.5 analog (100 mg, 0.13 mmol), HATU (47 mg, 0.13 mmol) and DIPEA (97 mg, 0.75 mmol) were added to 2 mL of anhydrous DMF. After 10 min, 2-(2-aminoethyl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione (115 mg, 0.37 mmol) was added. After 45 min, cold diethyl ether was added to the reaction and the precipitate was collected and dried under vacuum. The dry blue powder was dissolved in buffer A (water with 0.5% TFA) and purified on reverse phase HPLC (gradient 20-60% buffer B, ACN with 0.1% TFA). The pure fractions were collected and lyophilized. Yield: 77 mg, 62%. ¹H NMR (DMF-d₇): δ = 9.66 (t, 1H), 9.51 (s, 1H), 9.15 (d, 2H), 8.94 (d, 1H), 8.48 (d, 2H), 8.31 (t, 4H), 8.15 (t, 2H), 7.87 (t, 2H), 7.73 (t, 2H), 6.48 (s, 2H), 6.41 (b, 2H), 4.65 (t, 4H), 3.93 (t, 2H), 3.79 (t, 2H), 3.28 (s, 2H), 3.15 (m, 2H), 2.37 (t, 4H), 2.22 (s, 12H), 1.59 (m, 2H). [M-H]⁻ calcd for C₅₆H₅₇N₅O₉S₂: 1006.3598, found: 1006.4623.

Synthesis of the block copolymers proceeds as shown in figure below:
To a stirred solution of 1 (5.3 mg, 0.0210 mmol) and 2 (5.7 mg, 0.0105 mmol) in dry DMF (1784 µL) was added a solution of modified second generation Grubbs’ ruthenium catalyst (0.8 mg, 0.001 mmol) in dry DMF (50 µL). The reaction was left to stir under a N₂ atmosphere for 2 h. 86 µL were removed and quenched with ethyl vinyl for SEC-MALS analysis. A solution of the hydrophilic OEG-monomer, 3 (2.1 mg, 0.003 mmol) in DMF (100 µL) was added to the remaining reaction mixture. The mixture was left to stir under a N₂ atmosphere for additional 30 min, followed by ethyl vinyl ether quenching (5 µL). After 25 min the polymer was crashed out over cold ether to give an off-white solid.

Synthesis of polymer P0
To a stirred solution of 1 (15.5 mg, 0.0612 mmol) in dry DMF (3264 µL) was added a solution of modified second generation Grubbs’ ruthenium catalyst (1.5 mg, 0.002 mmol) in dry DMF (408 µL). The reaction was left to stir under a N₂ atmosphere for 2 h. 164 µL were removed and quenched with ethyl vinyl for SEC-MALS analysis. A solution of the hydrophilic OEG-monomer, 3 (4.2 mg, 0.006 mmol) in DMF (100 µL) was added to the remaining reaction mixture. The mixture was left to stir under a N₂ atmosphere for additional 30 min, followed by ethyl vinyl ether quenching (5 µL). After 25 min the polymer was crashed out over cold ether to give an off-white solid.

Synthesis of ¹⁵N and Cy5.5-labeled polymer (Cy⁻¹⁵N-P)

To a stirred solution of 1* (5.3 mg, 0.0210 mmol) and 2 (5.7 mg, 0.0105 mmol) in dry DMF (1784 µL) was added a solution of modified second generation Grubbs’ ruthenium catalyst (0.8 mg, 0.001 mmol) in dry DMF (50 µL). The reaction was left to stir under a N₂ atmosphere for 2 h. 86 µL were removed and quenched with ethyl vinyl for SEC-MALS analysis. A solution of the hydrophilic OEG-monomer, 3 (2.1 mg, 0.003 mmol) in DMF (100 µL) was added to the remaining reaction mixture and the mixture was left to stir under a N₂ atmosphere for additional 30 min.
living polymer was then split into two pots. While the first portion was quenched with ethyl vinyl ether, one equivalent of Cy5.5-monomer was added to the second portion and left to stir for 1 h before quenching with ethyl vinyl ether as well. The polymers were flashed out over cold ether. Mn, Mw and Đ of the first block determined by SEC-MALS were 1.55 g mol⁻¹, 1.86 x 10⁴ g mol⁻¹ and 1.20 respectively, thus indicating a degree of polymerization that can be denoted [1,2]₅₂. Mn, Mw and Đ of the block copolymer were determined to be 1.95 g mol⁻¹, 2.09 x 10⁴ g mol⁻¹ and 1.08, thus indicating the degree of polymerization of the final block and the denotation [1,2]₅₂⁻b⁻3₃ for the block copolymer.

**NP uptake analyzed via Structured Illumination Microscopy with membrane and nuclear staining**

HeLa cells were plated at 100,000 cells/dish in 35 mm glass-bottom cell culture dishes (precoated with fibronectin) 12 h prior to incubation with 500 µl of a 3 µM Cy-15N-NP suspension in DMEM for 4 h at 37 °C and 5% CO₂. After incubation, the media with the NPs was removed and fresh media with 5 µl of 1 mg/ml WGA-AF-488 was added and for 10 min incubation. After treatment, cells were incubated three times with heparin (15 U/ml in PBS) for 5 min at 37 °C and 5% CO₂ and washed twice with PBS. Cells were fixed for 15 min with 2% paraformaldehyde at room temperature and washed three times with PBS. 5 µl of DAPI (1 µg/ml) was added and incubated at 4 °C overnight. Cells were imaged in PBS with SIM. SIM imaging was performed on the Elyra S1 inverted fluorescence microscope (Zeiss). An oil immersion objective with 63X magnification and 1.4 numerical aperture was used in this study. 3 rotations and 5 phases were taken for each SIM image. The pixel size in the raw images was 80 nm per pixel, and in the resulting super resolution images was 40 nm per pixel. The camera exposure time was 100 ms per image. For every cell, 4 tracks were recorded sequentially for: (1) image the nucleus (DAPI) using 405 nm at 2.6 mW laser excitation and emission band 420 nm to 480 nm; (2) image the membrane (WGA) using 488 nm at 2.0 mW laser excitation and emission band 495 nm to 550 nm; (3) image the NPs (Cy5.5) using 642 nm wavelength at 3.5 mW power laser excitation and emission wavelength longer than 655 nm; (4) image the cell using bright-field transmission mode at a conventional resolution. The first 3 tracks were later processed to provide super resolution images by ZEN software (Zeiss).

**NP uptake and immunofluorescence analyzed via Structured Illumination Microscopy**

HeLa cells were plated at 100,000 cells/dish in 35 mm glass-bottom cell culture dishes (precoated with fibronectin) 12 h prior to incubation with 500 µl of a 3 µM Cy-15N-NP suspension in DMEM for 2 h at 37 °C and 5% CO₂. After incubation, the media with the NPs was removed and cells were washed twice with PBS. Cells were fixed for 15 min with 2% paraformaldehyde at room temperature and washed twice with PBS. Cells were permeabilized (0.5% saponin in PBS) for 15
Cells were incubated with blocking solution (0.2% saponin and 1% BSA in PBS) for 30 min. The primary antibodies mouse LAMP1 (ab25630, Abcam, Cambridge, MA) and rabbit EEA1 (ab2900, Abcam, Cambridge, MA) were added in a 1/100 and 1/800 dilution respectively in blocking buffer and incubated for 1 h. Cells were washed once with PBS and the secondary antibodies goat anti-mouse AlexaFluor488 (A11017, Invitrogen) and goat anti-rabbit AlexaFluor546 (A11071, Invitrogen) were added in a 1/1000 dilution in blocking buffer. 5 µl of DAPI (1 µg/ml) was added and incubated at 4 °C overnight.

Cells were imaged in PBS with SIM. The SIM imaging was performed as described above. For every cell, 4 tracks were recorded sequentially for: (1) image the NPs (Cy5.5) using 642 nm wavelength at 1 mW power laser excitation and emission wavelength longer than 655 nm; (2) image the endosomes (EEA1) using 561 nm at 8 mW laser excitation and emission band 570 nm to 620 nm; (3) image the lysosomes (LAMP1) using 488 nm at 2 mW laser excitation and emission band 495 nm to 550 nm; and (4) image the nucleus (DAPI) using 405 nm 2 mW laser excitation and emission band 420 nm to 480 nm. These tracks were later processed to obtain super resolution images using the ZEN software (Zeiss).

**NPs cellular trafficking analyzed via SIM and NanoSIMS**

HeLa cells were plated at 100,000 cells/dish in 35 mm culture dishes which had inside a 18 mm² ITO coverslip (70-100 Ω, 6462-AB, SPI supplies) precoated with fibronectin 12 h prior to incubation with 2 ml of a 15 µM Cy-15-N-NP suspension in DMEM for 4 or 24 h at 37 °C and 5% CO₂. After incubation, the media with the NPs was removed and fresh media with 10 µl of 1 mg/ml WGA-AF-488 (Life Technologies, W11261) and 2 drops of NucBlue (Hoechst33342, Life Technologies, R37605) were added and incubated for 10 min and 30 min, respectively. After treatment, cells were incubated three times with heparin (15 U/ml) for 5 min at 37 °C and 5% CO₂ and washed twice with PBS. Cells were fixed for 3 h with 2.5% glutaraldehyde at room temperature and washed for 15 min three times with PBS. The cells were then subjected to a series of dehydration washes with 30%, 50%, 75%, and 3 times with 100% ethanol (30 min each). The dry cells on the ITO glass were then first imaged by SIM followed by NanoSIMS.

The SIM imaging was performed as described above. For every cell, 4 tracks were recorded sequentially for: (1) image the NPs (Cy5.5) using 642 nm wavelength at 1 mW power laser excitation and emission wavelength longer than 655 nm; (2) image the membrane (WGA) using 488 nm at 0.4 mW laser excitation and emission band 495 nm to 550 nm; (3) image the nucleus (Hoechst) using 405 nm at 4.5 mW laser excitation and emission band 420 nm to 480 nm; (4) image the cell using bright-field transmission mode at a conventional resolution. The first 3 tracks were later processed to provide super resolution images by ZEN software (Zeiss). The X and Y coordinates of the sample stage were recorded for every image and were used to locate the cells for the following NanoSIMS imaging.
For the NanoSIMS analysis, the samples were coated with 10 nm of Au prior to analysis to minimize sample charging (Frisz et al. Proc. Natl. Acad. Sci. 2013, 288, 16855). High beam current sputtering was performed to remove ~15 nm of material from the surface of the sample and to assist in reaching sputter equilibrium (Woebken et al. ISME J. 2012, 6, 1427). After which, images with either 35 µm × 35 µm and 256 pixel × 256 pixel or 40 µm × 40 µm and 512 pixel × 512 pixel raster areas were acquired with a ~1 pA Cs⁺ primary ion beam (width ~115 nm) using magnetic peak switching, where in the first scan of a plane ¹²C⁻, ¹⁶O⁻¹H⁺, ¹²C¹⁴N⁻, and ¹²C¹⁵N⁻ were collected (1 ms/pixel) and in the second scan of the plane ¹²C¹⁵N⁻ and ¹⁹⁵Pt⁻ were collected (3 ms/pixel). Each scanning plane resulted in greater than 1 nm of removal and analysis (Ghosal et al. Anal. Chem. 2008, 80, 5986). Data was processed using OpenMIMS (National Resource for Imaging Mass Spectrometry, Harvard University, Cambridge), which is an ImageJ plugin (U. S. National Institutes of Health, Bethesda, Maryland). To determine the relative enrichment of ¹⁵N associated with Pt, the following steps were performed: (1) all planes of the ¹⁹⁵Pt⁻ ion image were summed to determine what regions ¹⁹⁵Pt⁻ was detected throughout the imaging experiment. (2) Regions of interested (5 pixels in size) were then selected that were and were not colocalized with the ¹⁹⁵Pt⁻ signal. (3) The ¹²C¹⁵N⁻/¹²C¹⁴N⁻ ratio was then determined for each of these ROIs, normalized to natural abundance ¹⁵N/¹⁴N (0.00367), and plotted as a function of image plane.
2. Time-dependent $^1$H NMR

Figure S1. Time-dependent $^1$H NMR (chemical shifts expressed in ppm), where the olefin peaks of the phenyl- and Pt(II)-monomer (a and c respectively) disappear within 2 h, confirming complete polymerization. It is important to note that since an amino group of the Pt(II)-monomer had a chemical shift similar to its olefin c protons, in order to observe their complete disappearance, deuterium exchange was carried out by adding a drop of D$_2$O to the NMR tube for analysis.
3. Size exclusion chromatography – multiangle light scattering plots

**Figure S2.** SEC-MALS plots expressed as relative intensity vs. time for the polymers obtained as the polymerization of monomers 1 and/or 2 and the subsequent polymerization of monomer 3 to give polymers P0, the four different batches of P and Cy-$^{15}$N-P.
4. Dynamic light scattering plots

Figure S3. Size distribution plots of NP₀, NP₇₁₄, and Cy⁻¹⁵⁻N-NP obtained by DLS and expressed as percent intensity vs. hydrodynamic diameter (nm) obtained for the dialysis of the polymers P₀, P₇₁₄ and Cy⁻¹⁵⁻N-NP from DMF into water.
Figure S4. Time-dependent NP stability. Top: NP$_{B4}$ as observed after being suspended in H$_2$O for 12 months. Bottom: time-dependent incubation in PBS (left) or serum-containing cell media (right). While the NPs size remains constant after incubation in PBS, size perturbations appear after incubation in cell culture media with serum on the time scale of 4 days.
5. NTA plots

Figure S5. Size distribution plots of NP0 and NP_{B1-4} obtained by NTA and expressed as n counts/µm vs. diameter (nm) obtained for the dialysis of the polymers P0 and P_{1-4} from DMF into water.
6. Transmission electron microscopy

Figure S6. TEM images of Pt(II)-loaded NPs: cryo TEM (top) and in situ TEM (bottom) (Proetto et al. JACS, 2014, 136, 1162).

Figure S7. Dry state TEM images of Pt(II)-loaded NPs after freezing and defrosting of the suspension, showing no morphological change.

Figure S8. Cryo TEM images of Pt(II)-loaded NPs incubated in water (left) and in cellular media in a time-dependent manner.
7. NP uptake analyzed via Structural Illumination Microscopy (SIM) with membrane and nuclear staining (companion to Figure 5, Main Text)

*Figure S9.* SIM analysis of the subcellular localization of **Cy-^{15}N-NP** (red) in HeLa cells examined using fluorescent probes. Cells were stained with a probe specific for the cell membrane (WGA-AF-488, green) and nucleus (DAPI, blue). Arrows show NP colocalized with endocytic vesicles that originated from the cell membrane as indicated by their green fluorescence.
8. NP uptake analyzed via SIM with lysosome, early endosome and nuclear staining (companion to Figure 5, Main Text)
**Figure S10.** SIM analysis of the subcellular localization of Cy-15N-NP (red) in HeLa cells examined using fluorescent probes specific for distinct cell organelles. Cells were double immunostained for early endosomes (EEA1-Alexa546, green) and lysosomes (LAMP1-Alexa488, blue) as well as for DNA (DAPI, white). Yellow arrows show NP colocalized with early endosomes and white arrows show NP colocalized with lysosomes.
9. NP (Cy-^{15}N-NP) uptake analyzed via NanoSIMS

Using the Cs\(^+\) primary ion as a probe for location specific ionization, the secondary ions of \(^{12}\text{C}^-\), \(^{16}\text{O}^-\text{H}^-\), \(^{12}\text{C}^{14}\text{N}^-\), and \(^{12}\text{C}^{15}\text{N}^-\) were collected in the first scan of the image plane, and then the magnetic field of the mass spectrometer was shifted to detect \(^{12}\text{C}^{15}\text{N}^-\) (not shown, but used to confirm no instrumental shift between scans) and \(^{195}\text{Pt}^-\) (3 ms/pixel) in the second scan of the plane. It is important to note, that unlike fluorescence microscopy, which is non-destructive and a z-stack of the sample is obtained (providing absolute three-dimensional information), the NanoSIMS technique is destructive and determines the composition of the sample surface (a so-called surface sensitive technique). One can sputter through a sample to gain three-dimensional information of the sample’s composition, but, due to the nature of desorption via an ion beam, z-information is not absolute across the imaging plane.
**Figure 11.** HeLa cell incubated with 15 µM of Cy-\(^{15}\)N-NP for 4 h and imaged by NanoSIMS. Removal of layers of organic matter from the cell surface followed by imaging provided a depth profile of the 5 different isotopes. The last column shows the colocalization (yellow) of the \(^{195}\)Pt (red) and enriched \(^{15}\)N (green) of the NP inside the cell. The cell surface is represented by the \(^{12}\)C-\(^{14}\)N' ion map (blue). This cell is the one shown in Figures 7 and 8 of the main text.

**Figure S12.** Further example of a HeLa cell incubated with 15 µM of Cy-\(^{15}\)N-NP for 4 h and imaged by NanoSIMS.
Figure S13. HeLa cell incubated with 15 μM of Cy-15N-NP for 24 h and imaged by NanoSIMS. Removal of layers of organic matter from the cell surface followed by imaging provided a depth profile of the 5 different isotopes. The last column shows the colocalization (yellow) of the 195Pt (red) and enriched 15N (green) of the NP inside the cell. The cell surface is represented by the 12C14N+ ion map (blue). This cell is the one shown in Figures 7 and 8 of the main text.
Figure S14. Further example of a HeLa cell incubated with 15 µM of Cy-\(^{15}\)N-NP for 24 h and imaged by NanoSIMS.
10. NP (Cy-$^{15}$N-NP) uptake analyzed via NanoSIMS

In an attempt to quantitatively demonstrate the colocalization of $^{15}$N and $^{195}$Pt within the cell, for both the 4 and the 24 h NP incubation times, we summed $^{195}$Pt signal of all planes to determine its location (images Below). From these summed images we created two sets of ROIs that were colocalized with the Pt and not colocalized with the Pt signal, but still within the cell boundaries. Finally the average $^{15}$N/$^{14}$N signal (obtained from the ratio of the $^{12}$C$^{15}$N/$^{12}$C$^{14}$N images) at different planes for those ROIs was determined and plotted.

![Figure S15. Summed $^{195}$Pt signal images of HeLa cells incubated for 4 (A) and 24 h (B). Shown in green and in red are the 5 pixel ROIs selected for $^{15}$N enrichment quantification, which were not or were respectively colocalized with the $^{195}$Pt signal.](image-url)
Figure S16. Average $^{12}\text{C}^{15}\text{N} / ^{12}\text{C}^{14}\text{N}$ counts (relative to natural abundance $^{15}\text{N} / ^{14}\text{N}$, NA) per previously selected ROI vs. number of plane on HeLa cells incubated with Cy-$^{15}\text{N}$-NP for 4 (A, left) and 24 h (B, right). Red and green traces correspond to ROIs, which were chosen on or off the $^{195}\text{Pt}$ signals respectively. It can be clearly observed that the $^{15}\text{N}$ enrichment signal is at least 10 greater in areas colocalized with Pt (on particle) than in areas not (off particle). It can also be observed that by increasing the plane number, which corresponds to areas deeper within the cell, the $^{15}\text{N} / ^{14}\text{N}$ signal generally becomes larger.
Control HeLa cells without NP treatment analyzed via NanoSIMS

Figure S17. HeLa cell incubated with 15 µM of Cy-\textsuperscript{15}N-NP for 0 h (Control cell 1), stained, washed and imaged by NanoSIMS. Removal of layers of organic matter from the cell surface followed by imaging provided a depth profile of the 5 different isotopes and the secondary electron image (SE, first column). The last column shows the overlay (yellow) of the \textsuperscript{195}Pt (red) and enriched \textsuperscript{15}N (green) of the NP inside the cell, and the cell surface is represented by the \textsuperscript{12}C\textsuperscript{14}N ion map (blue).
Figure S18. HeLa cell incubated with 15 µM of Cy-15N-NP for 0 h (Control cell 2), stained, washed and imaged by NanoSIMS. Removal of layers of organic matter from the cell surface followed by imaging provided a depth profile of the 5 different isotopes and the secondary electron image (SE, first column). The last column shows the overlay (yellow) of the 195Pt (red) and enriched 15N (green) of the NP inside the cell, and the cell surface is represented by the 12C14N- ion map (blue). For this image, high current sputtering between planes 8 and 9, and 12 and 13, where ~15 nm of material each time, was performed to remove cell material faster and demonstrate that no NP-based material was detected in the cell.
Figure S19. 20 ROIs were selected within the cell boundaries for each of the control cells 1 and 2. The plots represent the average $^{12}\text{C}^{15}\text{N}/^{12}\text{C}^{14}\text{N}^\text{xNA}$ counts (relative to natural abundance $^{15}\text{N}/^{14}\text{N},$ NA) and averaged $^{195}\text{Pt}$ per ROI vs. number of plane on HeLa cells incubated with Cy-$^{15}\text{N}$-NP for 0 h. It can be clearly observed that for both cells (cell 1, top and cell 2, bottom) no $^{15}\text{N}$ enrichment or $^{195}\text{Pt}$ was detected. One or two $^{195}\text{Pt}$ counts were observed in some ROIs, but their pattern was inconsistent, and is thus attributed to electron multiplier noise in the ion detection.
12. NP (Cy-^{15}N-NP) uptake analyzed via combined SIM and NanoSIMS

**Figure S20.** Individual SIM (Cy5.5, left) and NanoSIMS \((^{12}\text{C}^{15}\text{N}, ^{195}\text{Pt} \text{ and } ^{12}\text{C}^{14}\text{N} \text{ outline, middle})\) images together with the merged one (right). The top row corresponds to the cell incubated for 4 h and the bottom one for the cell incubated for 24 h. For the correlation on the 4 h cell (top), SIM plane number 10 and NanoSIMS plane 28 were used. For the correlation on the 24 h cell (bottom), SIM plane number 6 and NanoSIMS plane 54 were used. It is important to note, that in SIM, increasing plane number means farther away from the glass. In NanoSIMS, increasing plane number means closer to the glass.