Supporting Information for

Amphiphilic Polymeric Nanoparticles Enable Homogenous Rhodium-Catalysed NH Insertion Reactions in Living Cells

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1.1 Materials and Methods

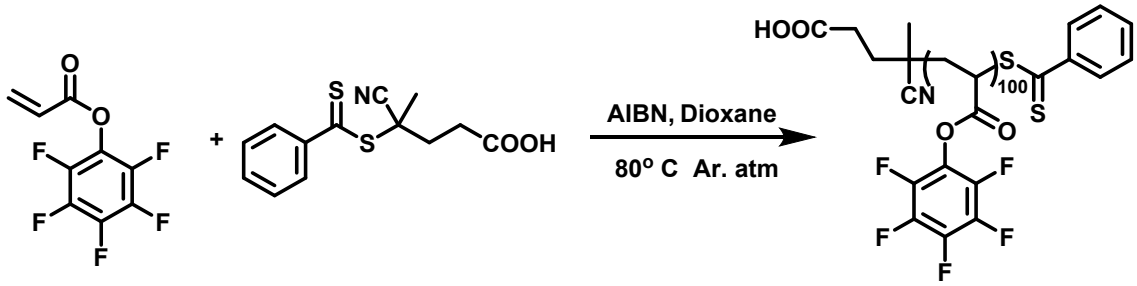
All NMR measurements were recorded on a Bruker 400 MHz spectrometer or Varian Mercury Vx 400 MHz (^{1}H at 400 MHz, ^{13}C at 100 MHz and ^{19}F at 376 MHz) at ambient temperature. The ^{1}H NMR chemical shifts (*d*) are reported in ppm downfield from tetramethylsilane (TMS). The resonance of the deuterated solvent was used as an internal standard for reporting ^{13}C NMR chemical shifts (*d*). Peak multiplicities are abbreviated as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Downfield ^{13}C NMR chemical shifts are reported using the resonance of the deuterated solvent as an internal standard. Br = broad, d = doublet, dd = double doublet, m = multiplet, p = pentet, s = singlet, t = triplet, and q = quartet are the abbreviations used. Shimadzu Prominencei LC2030C 3D equipped with a Shimadzu RID20A refractive index detector was used to perform SEC measurements. The elution solvent used was THF containing 10 mM LiBr (flow = 1 mL/min). A mixed C and mixed D column combined in series with an exclusion limit = 2.000.000 g mol-1, 7.5 mm i.d. 300 mm previously calibrated with poly(styrene) from Polymer Laboratories was used. DMF-SEC measurements were carried out on a PLGPC50 plus (Varian) equipped with a refractive index detector. Dimethylformamide containing 10 mM LiBr (flow = 1 mL min^{-1}) was used as eluent in combination with a Shodex GPCKD804 column (exclusion limit = 400.000 Da; 0.8 cm i.d. 300 mm). This column was calibrated with poly(ethylene glycol) from Polymer Laboratories. Dialysis was carried out with the help of a Spectra/Por Dialysis Membrane with a Molecular Weight CutOff of 6-8 kDa. Malvern Zetasizer with 830 nm laser at 90° was used for dynamic light scattering experiments and samples were filtered over a 0.2 µm PVDF filter prior to measurements to remove any dust particles from hindering the measurements. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) measurements were performed on a SpectroBlue Ametek after calibration using rhodium standard solution at different concentrations. Grace Reveleris X2 was utilized for automated column chromatography where Büchi prepacked silica columns were used to load sample (liquid loading/dry loading as specified). High Performance Liquid chromatography LC-UV was performed using Shimadzu UFLC-XR with PDA detector. Eluents used were A: water + 0.1% formic acid and B: ACN + 0.1% formic acid. The column used was a Kinetex column EVO C18, with length 50 mm and internal diameter 2.1 mm. It has a pore size of 100 Å and particle size 5 µm. Optimised HPLC method is as follows: A/B (90:10) isocratic 5 min, followed by gradient A/B (90:10) to A/B (0:100) in 2 min, A/B (0:100) isocratic 2 min, A/B (0:100) to A/B (90:10) gradient in 2 min. The flow rate was kept constant at 0.2 mL/min and measurements were performed at room temperature. High Performance Liquid Chromatography – HPLC-UV/MS was performed on a SHIMADZU Nexera-I LC-2040C 3D coupled with LC-MS 2020 for detection. Eluents, column and HPLC method for separation was the same as that used for HPLC-UV, except that the flow rate was kept constant at 0.15 mL/min and the column temperature was maintained at 40 °C. For cell experiments, analysis of the microplate was performed using a Tecan MC-SPARK. Agilent Cary Eclipse fluorescence spectrophotometer coupled with a temperature controller was used fluorescence kinetic experiments. Confocal microscopy images of HeLa cells were obtained with a Leica SP5 confocal microscope with a HyD2 detector. Identical conditions were followed throughout all measurements. Leica software and ImageJ was used for processing images. Amicon® Ultra-15 Centrifugal Filter Units

were used for filtering polymers from catalysts. Rhodium ICP standard solution was purchased from Sigma Aldrich and was diluted in deionized water to prepare respective concentration ranges.

All commercial reagents were purchased from Sigma Aldrich or TCI and were used without further purification. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermofisher Scientific. CCK-8 viability assay kit was purchased from Sigma-Aldrich. All bulk solvents were purchased from Biosolve. HeLa cells (ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10%v/v fetal bovine serum (FBS) and 1% v/v penicillin and streptomycin. After HeLa cells reach 70% confluence in the flask, cells were seeded in the 96 well plate with a cell density of 8000 cells/well. The plate was then placed in an oven at 37 °C with 5% CO_2 flow for 24 hours before adding substrate/products/catalytic nanoparticles. All the deuterated solvents were purchased from Cambridge Isotopes Laboratories. Dry solvents were obtained from an MBRAUN Solvent Purification System (MBSPS). Jeffamine M1000 polyetheramine was kindly provided by Huntsman. All catalysts were commercially available and used as is. Diazo substrates **2a-c** and corresponding products **3a-c** were synthesized according to the reported protocol.[1,2]

1.2 Synthesis

*1.2.1 RAFT polymerization of pentafluorophenyl acrylate (**p-PFPA**)*[3]



Pentafluorophenyl acrylate (6.3 mmol, 1.5 gram), 2,2'-azobis(2-methylpropionitrile) (AIBN) (4.4 µmol, 0.724 mg) and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (44 µmol, 12.3 mg) were dissolved in anhydrous dioxane (2 mL). The reaction mixture was degassed by Argon bubbling for 1 h. The reaction flask was kept under argon atmosphere through the remainder of the polymerization. The polymerization was started by placing the reaction flask in a pre-heated oil bath (80 °C). Conversion was monitored by ^{19}F-NMR. The polymerization was quenched in liquid nitrogen after approximately 4.5 h, when the conversion reached 72 %. Chloroform was added to the thawed reaction mixture and the reaction mixture was precipitated in cold n-pentane (~900 mL). The formed pink precipitate was filtered out and subsequently redissolved in chloroform. An extra 4 precipitations were performed to remove unreacted pentafluorophenyl acrylate. Finally, ***p*-PFPA** was dried in vacuo at 50 °C overnight and obtained as a pink solid.

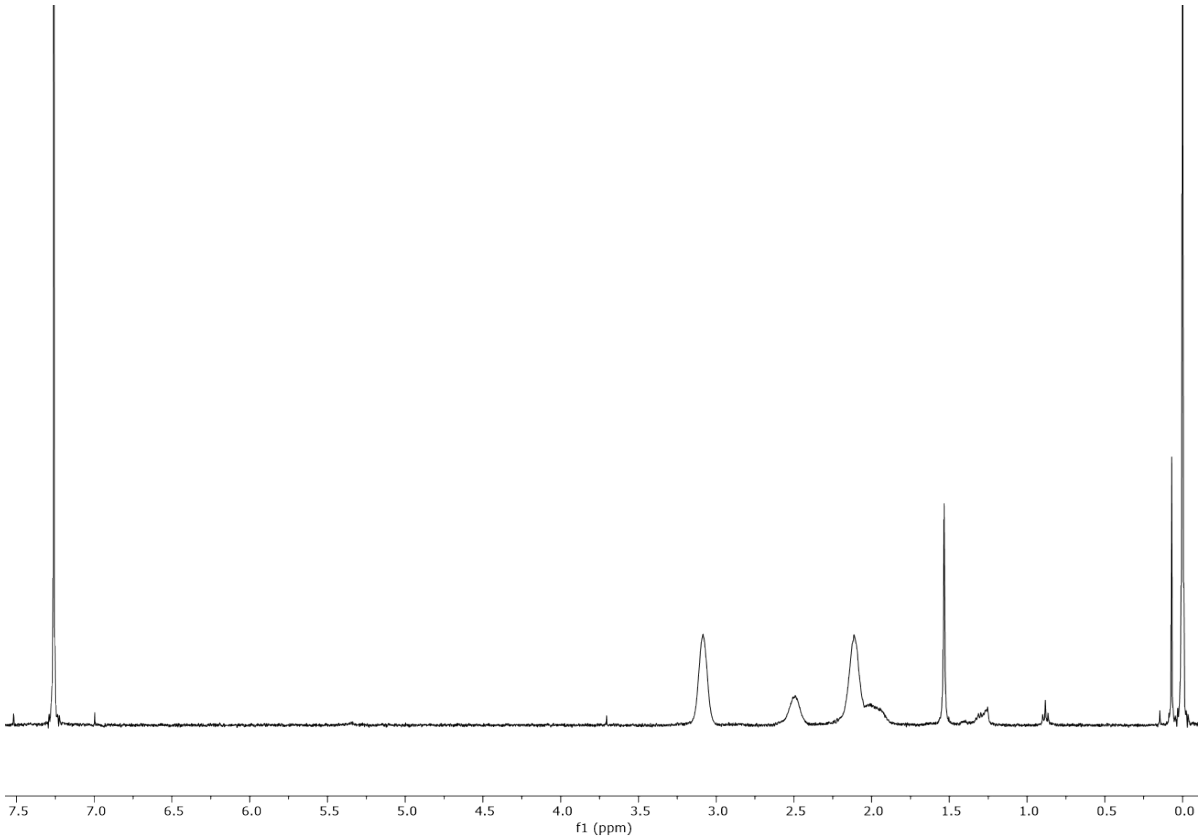


Figure S1: The ^{1}H-NMR spectrum of ***p*-PFPA** in $CDCl_3$.

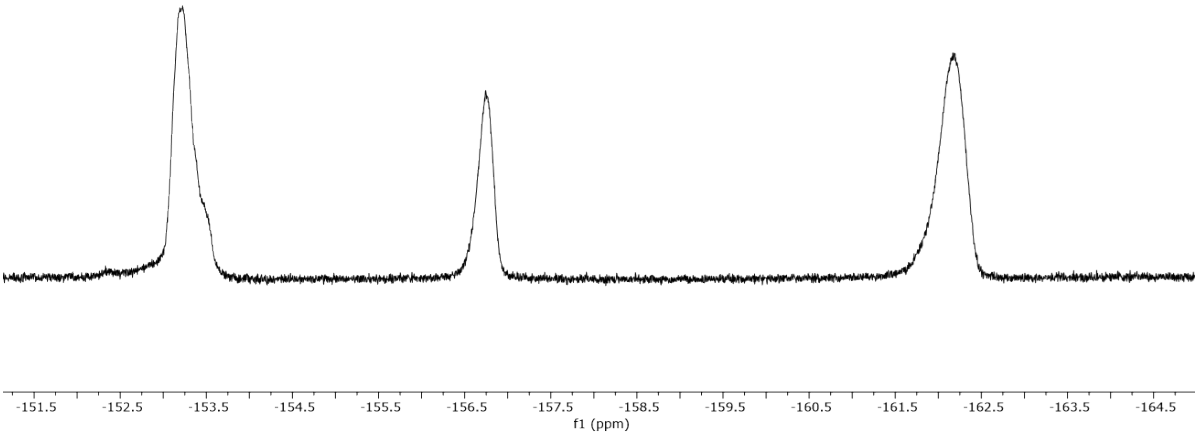


Figure S2: The ^{19}F-NMR spectrum of ***p*-PFPA** in $CDCl_3$ after precipitation.

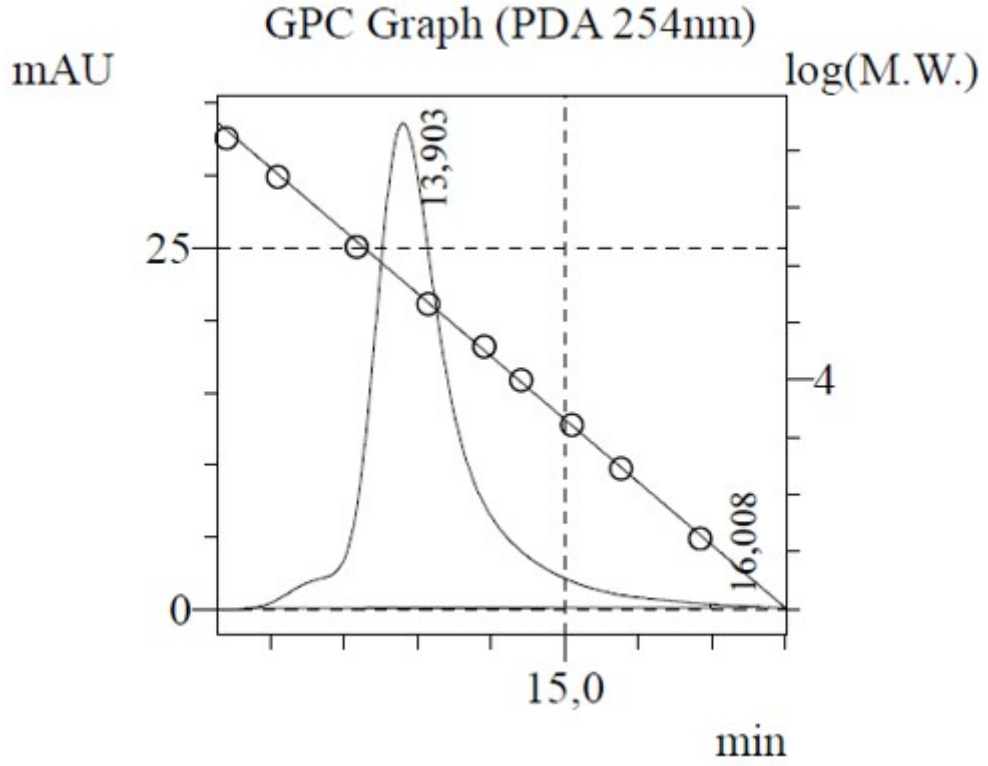
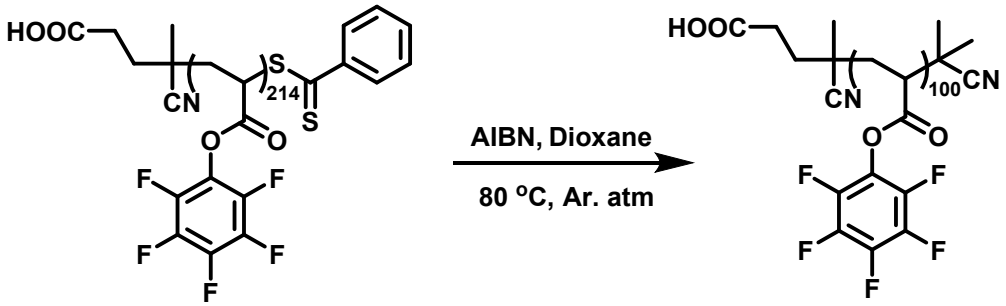


Figure S3: The GPC trace of ***p*-PFPA** in THF.

*1.2.2 End-group modification of poly(pentafluorophenyl acrylate) (**p-PFP**)*[3]



Polymer ***p*-PFPA** (16.3 µmol, 401 mg), 2,2'-azobis(2-methylpropionitrile) (AIBN) (0.33 mmol, 53.7 mg) and lauroyl peroxide (32.7 µmol, 13.0 mg) were dissolved in anhydrous dioxane (9 mL). The reaction mixture was degassed by Argon bubbling for 45 minutes. The reaction mixture was then placed in a pre-heated oil bath (80 °C) while stirring vigorously. The reaction was quenched after approximately 3.5 hours when the reaction mixture had turned colourless. Solvent was removed under reduced pressure and the polymer was redissolved in DCM and purified via precipitation in cold n-pentane (2x). ***p*-PFP** was obtained as a white solid.

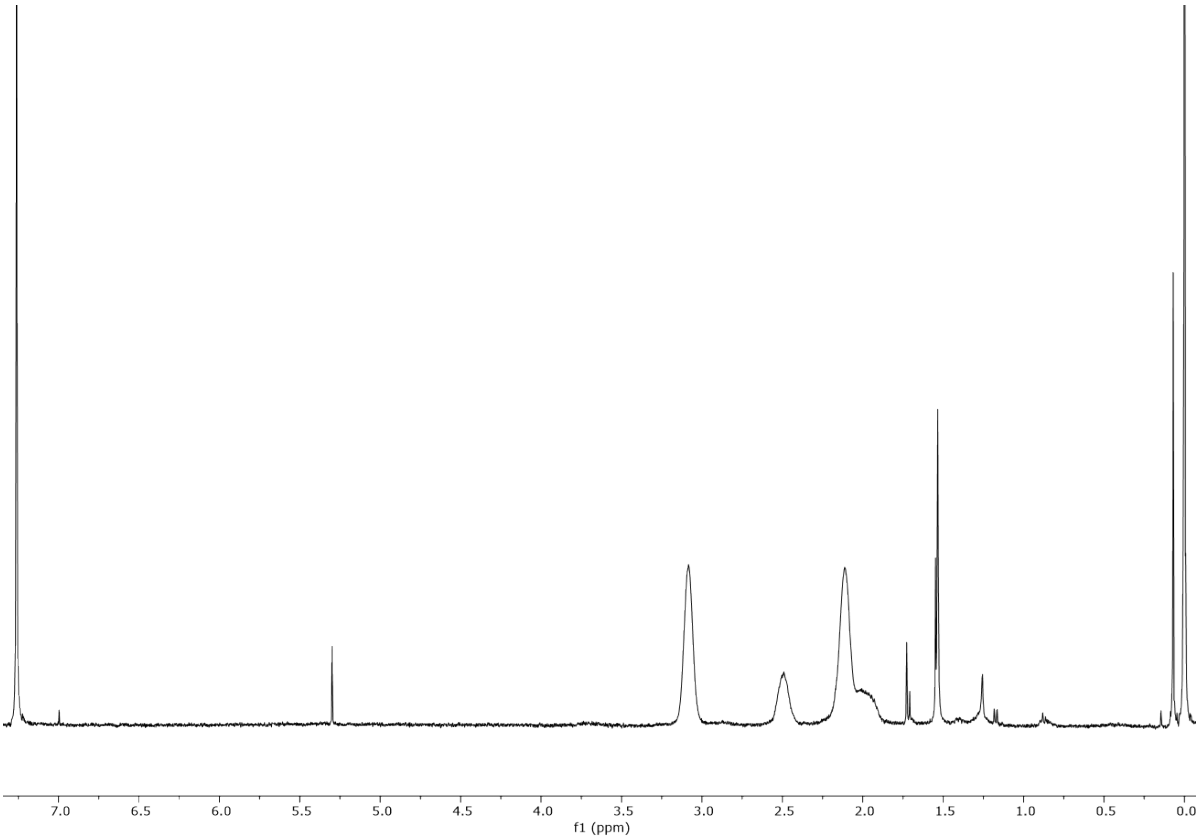


Figure S4: The ^{1}H-NMR spectrum of ***p*-PFP** in $CDCl_3$

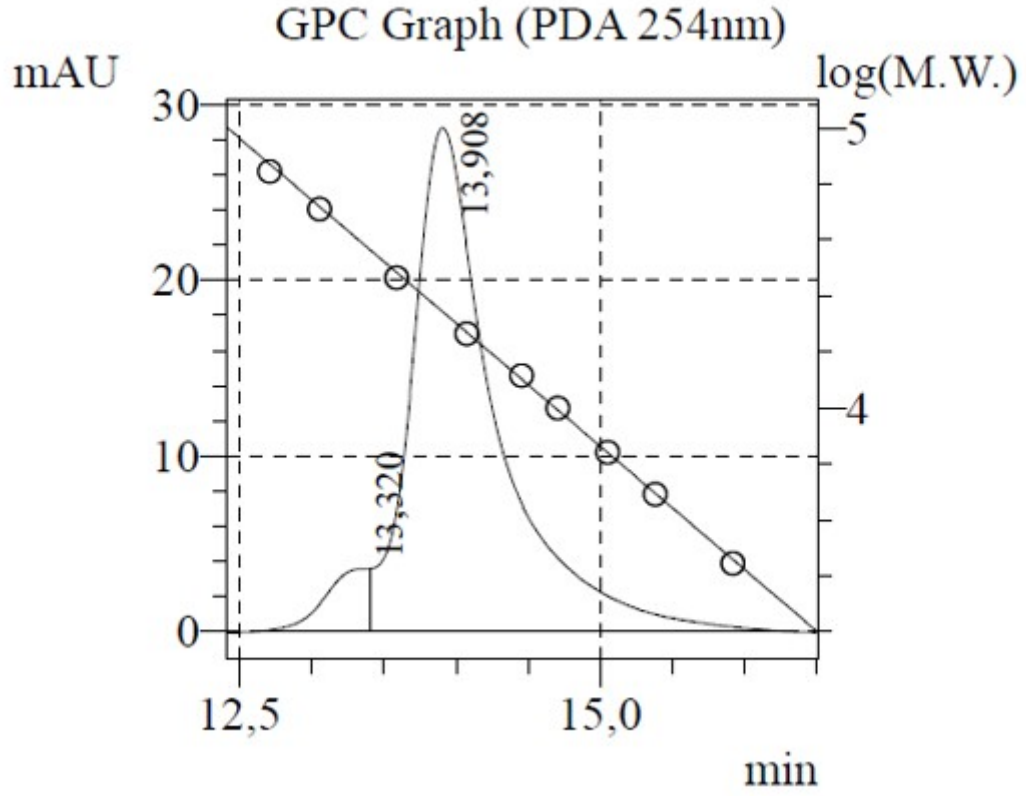


Figure S5: The GPC trace of ***p*-PFP** in THF.

*1.2.3 Post-functionalization of poly(pentafluorophenyl acrylate) (**P1**)*[3]

Polymer ***p*-PFP** (7.78 µmol, 190.3 mg, 1.0 eq) was dissolved in dry THF. Dodecyl amine (0.156 mmol, 26.6 mg, 20 eq.) was dissolved in dry THF and added to the polymer solution. The reaction mixture was immersed in a pre-heated oil-bath (50 °C) and stirred vigorously under argon atmosphere for 2 hours. Next, an excess Jeffamine M-1000 (1.75 mmol, 1.75 gram, 225 eq.) dissolved in dry THF was added to the reaction mixture. The reaction mixture was stirred at 50 °C overnight. The reaction mixture was first dialyzed against THF and subsequently against MeOH. Finally, the reaction mixture was precipitated in cold *n*-pentane and **P1** was dried under vacuum at 50 °C.

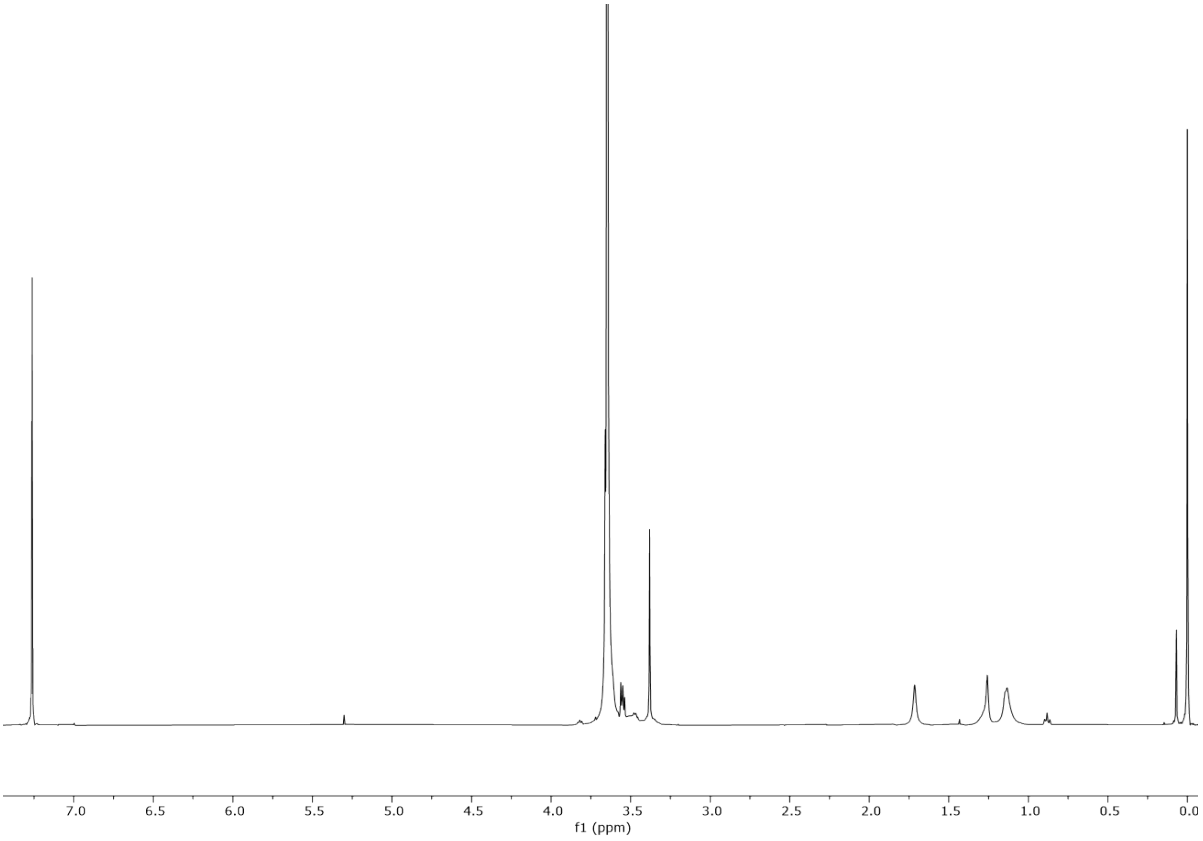


Figure S6: The ^{1}H-NMR of **P1** in $CDCl_3$

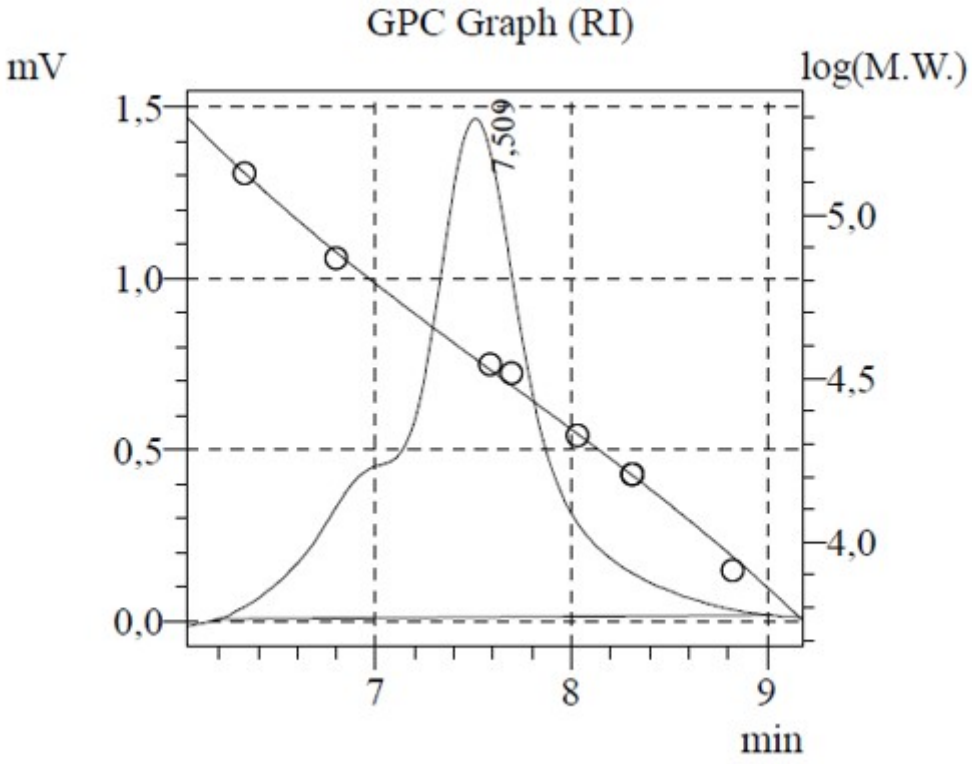


Figure S7: The GPC trace of **P1** in DMF.

*1.2.4 Synthesis of diazo substrates **2a-c** and benzoquinoxalines **3a-c***

*Synthesis of 2-diazo-3-oxo-3-phenylpropanoate (**2a**)*

Ethyl benzoylacetate (0.45 mL, 2.6 mmol, 1.0 eq.) was added to a solution of p-acetamidobenzenesulfonyl azide (p-ABSA, 0.75 g, 3.12 mmol, 1.2 eq.) in dry ACN (5 mL) at 0 °C under argon atmosphere. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 0.47 mL, 3.12 mmol, 1.2 eq) was then added dropwise resulting in a clear yellow solution. The reaction mixture was stirred for 5h and then slowly brought to room temperature. The reaction mixture was quenched with water (6 mL) and extracted with ethyl acetate (3 x 6 mL). The combined organic phases were dried over sodium sulfate, filtered under gravitation and concentrated under reduced pressure. By means of silica flash chromatography using heptane/ethyl acetate (95/5) as eluent, the pure product was obtained as yellow oil. ^{1}H-NMR was in concordance with literature.[2] Yield: 76% (431.00 mg, 1.98 mmol) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 – 7.57 (m, 2H), 7.57 – 7.47 (m, 1H), 7.47 – 7.35 (m, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H).

*Synthesis of ethyl 2-diazo-3-oxobutanoate (**2b**)*

Ethyl acetoacetate (0.34 mL, 2.7 mmol, 1.0 eq.) was added to a solution of p-acetamidobenzenesulfonyl azide (p-ABSA, 0.75 g, 3.13 mmol, 1.2 eq.) in dry ACN (5.4 mL) at 0 °C under argon atmosphere. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 0.48 mL, 3.13 mmol, 1.2 eq) was then added dropwise resulting in a yellow thick paste. An additional 0.5 mL dry ACN were added and the clear orange reaction mixture was stirred for 6h. Upon completion as indicated by TLC, the reaction mixture was slowly brought to room temperature, quenched with water (6 mL) and extracted with ethyl acetate (3 x 6 mL). The combined organic phases were dried over magnesium sulfate, filtered under gravitation and concentrated under reduced pressure. By means of silica flash chromatography using heptane/ethyl acetate (9/1) as eluent, the pure product was obtained as yellow oil. ^{1}H-NMR was

in concordance with literature.[2] Yield: 65% (272 mg, 1.74 mmol) ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.31 (q, *J* = 7.1 Hz, 2H), 2.48 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H).

*Synthesis of 2-diazo-acetophenone (**2c**)*

Methylamine (40% in MeOH, 0.29 mL, 2.83 mmol, 0.9 eq.) was added dropwise to a solution of p-acetamidobenzenesulfonyl azide (p-ABSA, 0.75 g, 3.12 mmol, 1.0 eq.) and benzoylacetone (0.51 g, 3.12 mmol, 1.0 eq.) in ethanol (3.2 mL). Upon addition of methylamine the reaction mixture turned orange and into a thick paste. To restore stirring, 10 mL of ethanol were added. The now yellow reaction mixture was left to stir for 2 h at room temperature under argon atmosphere. Upon completion as indicated by TLC, the reaction mixture was concentrated under reduced pressure. The pure product was obtained by silica flash chromatography with heptane/ethyl acetate (9/1) as eluent as an orange solid. ^{1}H-NMR was in concordance with literature[2]. Yield: 70% (317 mg, 2.17 mmol) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.70 (m, 2H), 7.59 – 7.50 (m, 1H), 7.45 (dd, *J* = 8.3, 6.8 Hz, 2H), 5.90 (s, 1H).

*Synthesis of 3-phenylbenzo[g]quinoxaline-2-carboxylate (**3a**)*

To a 4.0 mL vial containing a stirrer and diazocarbonyl **2a** (22.1 mg, 0.1 mmol, 1.0 eq.) a solution of $Rh_2(esp)_2$ (10 mol%) in Milli-Q water (250 µL) was added. To ensure the complete transfer of the heterogenous catalyst $Rh_2(esp)_2$, the vial was washed again 3 times with 250 µL of Milli-Q water and added to the reaction mixture. 2,3-diaminonaphthalene (15.6 mg, 0.1 mmol, 1.0 eq.) was subsequently added to reaction mixture. The heterogenous reaction mixture was placed in a pre-heated oil bath (40 °C) and left to stir for 24 h, open to air. The reaction mixture was transferred to a 16 mL vial and extracted with ethyl acetate (4x 3 mL). The combined organic phases were dried over magnesium sulfate, filtered under gravitation and dried under reduced pressure. The pure product was obtained by column separation on the Grace Column (12 gr, 20 µm Silica), using heptane/EtOAc (9:1) as eluent. Due to the homoeopathic scale of reaction, yield could not be calculated. ^{1}H-NMR is in concordance with literature.[2] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H), 8.77 (s, 1H), 8.21 – 8.08 (m, 2H), 7.88 – 7.75 (m, 2H), 7.70 – 7.58 (m, 2H), 7.54 (dd, *J* = 5.1, 1.9 Hz, 3H), 4.37 (q, *J* = 7.2 Hz, 2H), 1.21 (t, *J* = 7.1 Hz, 3H).

*Synthesis of 3-methylbenzo[g]quinoxaline-2-carboxylate (**3b**)*

To a 16 mL vial containing a stirrer and diazocarbonyl **2b** (15.70 mg, 100 µmol, 1.0 eq.), a solution of $Rh_2(OAc)_4$ (10 mol%) in Milli-Q water (1.0 mL) was added. 2,3-diaminonaphthalene (15.70 mg, 100 µmol, 1.0 eq.) was then added and the reaction mixture was placed in a pre-heated oil-bath at 40 °C and stirred for 24 h open to air. Then, 1.5 mL of Milli-Q water was added and the reaction mixture was extracted with ethyl acetate (3x 5 mL). The combined organic phases were dried over magnesium sulfate, filtered under gravitation and concentrated under reduced pressure. The product was obtained by column separation on the Grace Column (4 gr, 20 µm Silica) using heptane/ethyl acetate (8/2) as eluent. Due to the homoeopathic scale of reaction, yield could not be calculated. ^{1}H-NMR is in concordance with literature.[2] ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H), 8.61 (s, 1H), 8.12 (dd, *J* = 7.8, 1.9 Hz, 2H), 7.72 – 7.47 (m, 2H), 4.59 (q, *J* = 7.2 Hz, 2H), 2.99 (s, 3H), 1.52 (t, *J* = 7.1 Hz, 3H).

*Synthesis of Tyrphostin AG1385 (**3c**)*

To a 16 mL vial containing 2,3-diaminonaphthalene (16.0 mg, 101 µmol, 1.0 eq.), $Rh_2(OAc)_4$ was added. Then 0.8 mL of Milli-Q water was added and the mixture was placed in a pre-heated oil (40 °C) while stirring open to air. Diazocarbonyl **2c** (14.8 mg, 101 µmol, 1.0 eq.) was dissolved in 0.2 mL MeCN and added slowly over 4 h with a syringe pump. After 24 h, 1.5 mL of Milli-Q water were added and the reaction mixture was extracted with ethyl acetate (4x 5 mL). The combined organic phases were dried over magnesium sulfate, filtered under gravitation and concentrated under reduced pressure. The product was obtained by column separation on the Grace Column (4 gr, 20 µm Silica) using a gradient from heptane to heptane/ethyl acetate (9:1) as eluent. ^{1}H-NMR is in concordance with literature.[2] Due

to the homoeopathic scale of reaction, yield could not be calculated. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.72 (d, *J* = 16.2 Hz, 2H), 8.34 – 8.19 (m, 2H), 8.20 – 8.09 (m, 2H), 7.67 – 7.51 (m, 5H).

1.3 Catalyst encapsulation in amphiphilic polymeric nanoparticles

1.3.1 General procedure for the encapsulation of dirhodium-based catalyst in amphiphilic polymer

Depending on the encapsulation ratio (**P**:**C**) or variability in the experiment, the required amounts of polymer and catalyst were co-dissolved in trichloroethylene and transferred to a glass vial. Trichloroethylene was gently allowed to evaporate by nitrogen or argon flow to form a thin film on glass, and this film was dried completely under vacuum at 60 °C overnight. To this vial, Milli-Q water was added, vortexed, and sonicated for 45 min. After sonication, the solution was placed in a pre-heated oven at 80 °C for 45 min. The solution was then suspended in air overnight to equilibrate to room temperature.

1.3.2 Procedure for determining the encapsulation efficiency

10 mL of catalyst-encapsulated polymeric nanoparticles **P1@C1-C4** were prepared according to the protocol described above, with polymer:catalyst ratio = 1:5, such that the solution has 10 µM **P1** and 50 µM **C1-C4**. Rhodium calibration curves were created using rhodium standard solution from a series of concentrations 0.1 – 0.5 mg/mL and 2-10 mg/mL separately. Calibration curves at these two concentration regimes were created each time before quantification for better accuracy. Catalyst-encapsulated nanoparticles **P1@C1-C4** were added to centrifugal filters with a molecular weight cutoff of 50 kD and were centrifuged for 15 min at 3000 RPM. The filtrate solution was then analysed using ICP-OES to determine the concentration of rhodium leached out from polymeric nanoparticles into water.

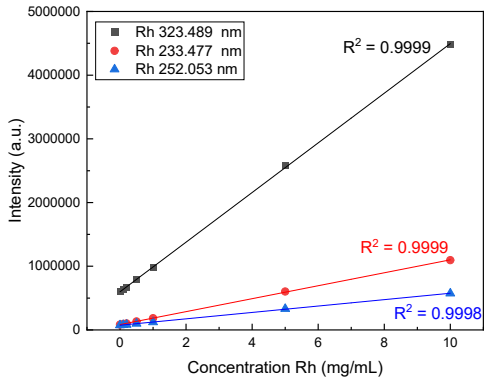


Figure S8: Calibration curves of Rh standard for ICP-OES measurements

Concentration Rh (mg/mL)	I@323.489 nm (a.u.)	I@233.477 nm (a.u.)	I@252.053 nm (a.u.)
0	597028	83952	73665.3
0.1	631413	93193.2	78368.7
0.2	668792	102229	82691.4
0.5	797329	133656	98850.5
1	980946	183987	121903
5	2585620	603489	331219
10	4477100	1095440	573159

1.4 Dynamic light scattering measurements

Samples for dynamic light scattering experiments were prepared according to the previously described procedure, where amphiphilic polymeric nanoparticles were prepared in MilliQ water at 1 mg/mL concentration, with different catalyst **C1** incorporation ratio ranging from **P1**:**C1** = 1:0 to 1:10 (molar ratio).

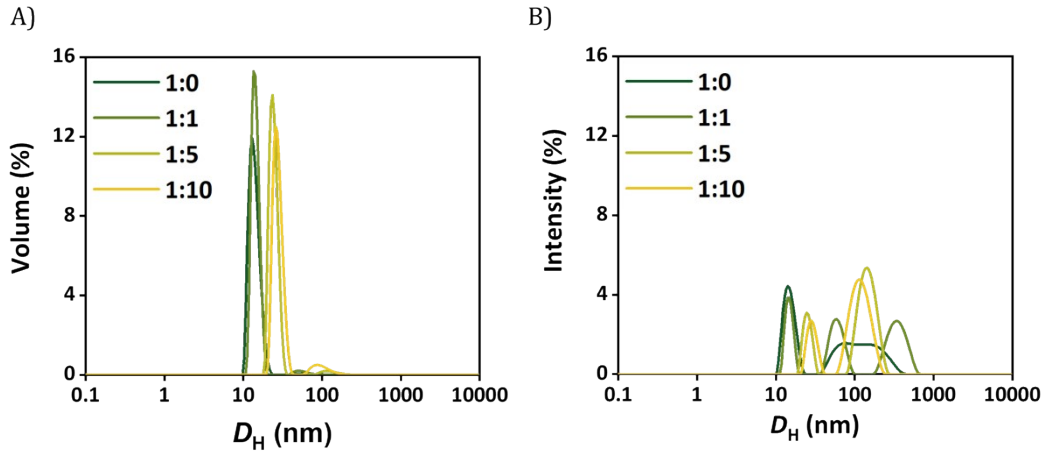


Figure S9: Dynamic light scattering analysis of **P1** and **P1@C1** at different catalyst incorporation ratios A) Volume plot; B) Intensity plot; [**P1**] = 1 mg/mL, in H_2O at room temperature, ratio represents molar ratio **P1**:**C1** ranging from 1:0 to 1:10.

1.5 NH carbene insertion reactions in aqueous solutions

*1.5.1 General procedure for catalytic NH-insertion reactions in presence of **P1:***

Water or PBS as reaction medium: To a solution of catalyst-encapsulated nanoparticles **P1@C1-C3** (10 µM **P1**, **P1**:**C** = 1:5), which was prepared in 992 µL Milli-Q water/PBS, 4 µL diamine **1** (0.5 µmol, 1.0 eq.) was added from a stock solution in DMSO (125 mM). The reaction mixture was stirred briefly before the addition of **2a** (4 µL, 0.5 µmol, 1.0 eq) from a stock solution in DMSO (125 mM). The reaction mixture was placed in a pre-heated oil bath (37 °C) and vigorously stirred for 24 h, open to air. After each required time point, a sample aliquot was taken. 10 µL aliquot was added to a vial to which 40 µL Milli-Q water and 50 µL acetonitrile was added. The sample was vortexed and then injected into HPLC-UV/MS. Although this procedure represents reactions with 500 µM substrate concentration, for other reactions where substrate and catalyst concentrations were varied, the same protocol was followed.

DMEM or DMEM+FBS: the same procedure as described above was followed with the exception that **P1@C1-C3** was added from a concentrated stock solution in Milli-Q water (500 µM) which was prepared according to the encapsulation procedure described above. The final [**P1@C1-C3**] was 10 µM (**P1**), where concentration of **C1-C3** was 50 µM.

*1.5.2 General procedure for catalytic NH-insertion reactions in absence of **P1:***

Water or PBS or DMEM or DMEM/FBS as reaction medium: 988 µL corresponding medium was added to a glass vial, followed by the addition of **1** and **2a** (from corresponding stock solution), same concentration as mentioned above. Water soluble catalyst **C4** was added to this mixture from a stock solution of 12.5 mM in THF in such a way that the final concentration of the catalyst is 50 µM and

substrates are 500 µM in the reaction medium. The reaction mixture was placed in a preheated oil bath at 37 °C and was vigorously stirred for 24 h. During the reaction, at different time points, aliquots were taken and diluted in water and acetonitrile as mentioned above. This solution was then injected into HPLC-UV/MS for monitoring the reaction progress.

For control experiments with hydrophobic catalyst **C1**, same procedure as above was followed where **C1** was directly added from a 12.5 mM stock solution in DMSO.

*1.5.3 Formation of product **3a** – reaction between **1** and **2a***

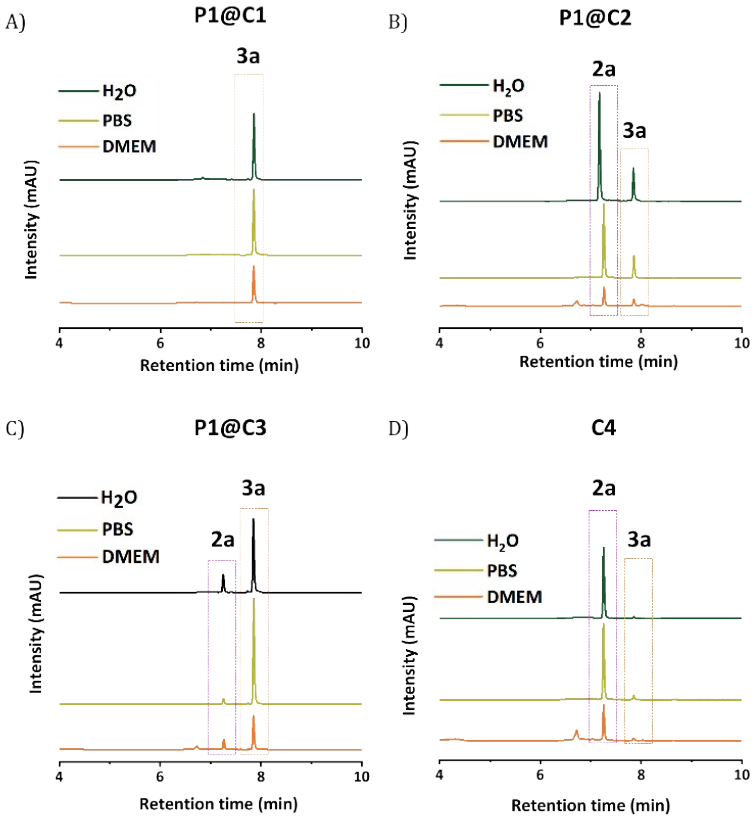


Figure S10: HPLC-UV chromatogram of the reaction progress of **1** and **2a** after 24 h. The reaction was catalysed by A) **P1@C1**; B) **P1@C2**; C) **P1@C3**; D) **C4**, monitored at 279 nm. Reaction conditions: [**1**] = [**2a**] = 500 µM, [**C**] = 50 µM, [**P**] = 1 mg/mL, **P1**:**C1-C3** = 1:5 (molar ratio), at 37 °C in water, PBS or DMEM medium.

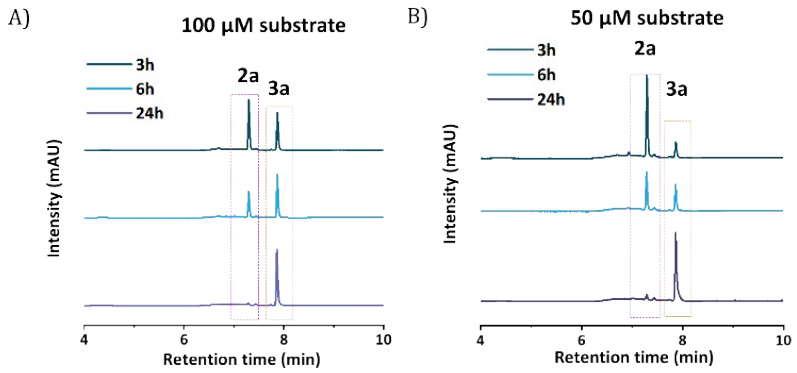


Figure S11: HPLC-UV chromatogram of the reaction progress of **1** and **2a** over time catalysed by **P1@C1**, monitored at 279 nm. A) [**1**] = [**2a**] = 100 µM, [**C1**] = 10 µM in water; B) [**1**] = [**2a**] = 50 µM, [**C1**] = 5 µM in water; All reactions are performed at 37 °C, with [**P1**]:[**C1**] = 1:5 (molar ratio).

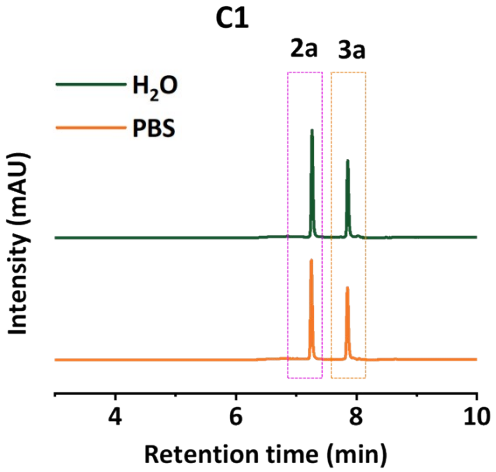


Figure S12: HPLC-UV chromatogram of the reaction progress of **1** and **2a** after 24 h catalysed by **C1** monitored at 279 nm. Reaction conditions: [**1**] = [**2a**] = 500 µM, [**C1**] = 50 µM, [**P1**] = 10 µM, at 37 °C in H_2O and PBS.

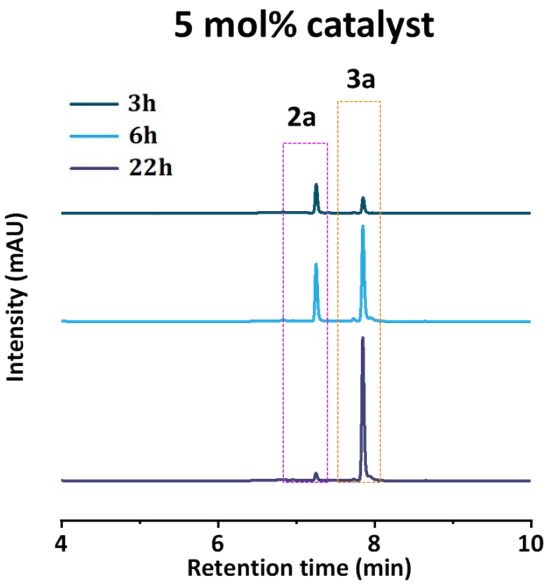


Figure S13: HPLC-UV chromatogram of the reaction progress of **1** and **2a** over time catalysed by **P1@C1**, monitored at 279 nm, [**1**] = [**2a**] = 500 µM, [**C1**] = 25 µM in water, [**P1**] = 5 µM, at 37 °C, with [**P1**]:[**C1**] = 1:5 in H_2O.

*1.5.4 Formation of product **3b** and hydrolyzed product **3b'**– reaction between **1** and **2b***

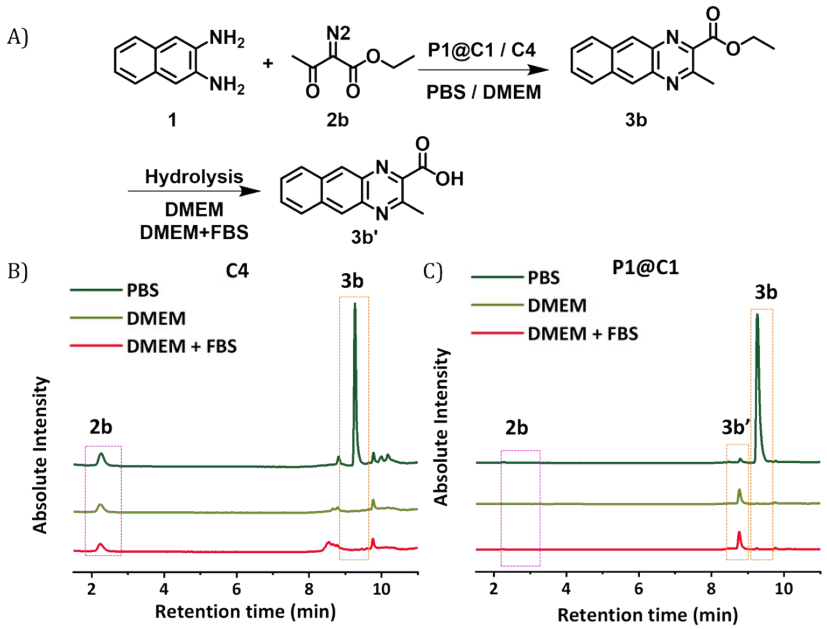


Figure S14: A) Formation of benzoquinoxaline hydrolysed product **3b'** from **3b**, when **1** and **2b** was reacted with **P1@C1**; B) HPLC chromatogram of the same reaction catalysed by **C4**; C) by **P1@C1** after 24 h, monitored at 279 nm wavelength. [**1**] = [**2b**] = 500 µM in PBS, DMEM and DMEM+10% FBS, [**C1**] = 50 µM, all reactions performed at 37 °C, [**P1**]:[**C1**] = 1:5, [P] = ~ 1 mg/mL.

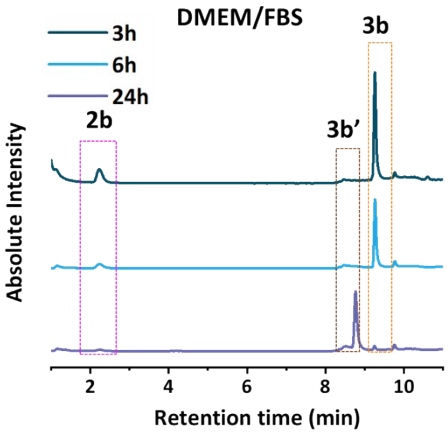


Figure S15: HPLC chromatogram of the same reaction catalysed by **P1@C1** overtime, monitored at 279 nm wavelength. [**1**] = [**2b**] = 500 µM in DMEM+10% FBS, [**C1**] = 50 µM, all reactions performed at 37 °C, [**P1**]:[**C1**] = 1:5 (molar ratio), [**P1**] = 10 µM.

*1.5.5 Formation of product **3c** – reaction between **1** and **2c***

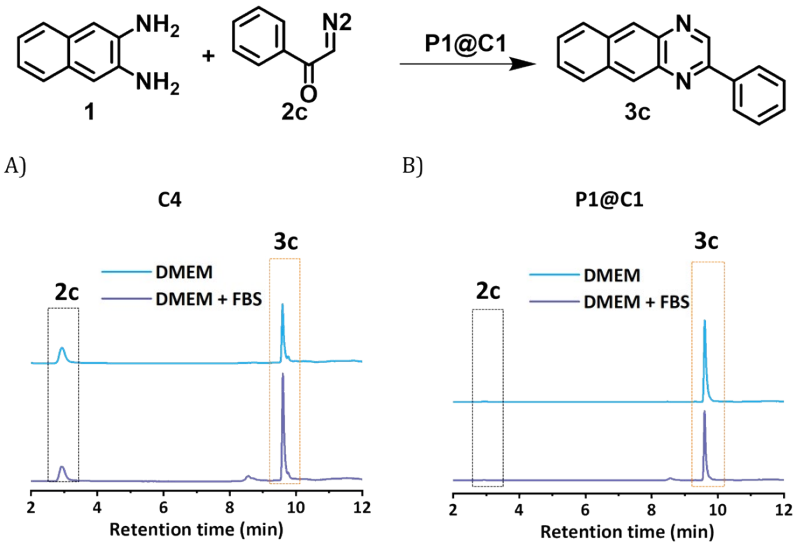


Figure S16: Formation of benzoquinoxaline **3c** when **1** and **2c** was reacted with **P1@C1** or **C4**; A) HPLC-MS chromatogram of the reaction catalysed by **C4**; B) by **P1@C1** after 24 h, monitored at 279 nm. [**1**] = [**2c**] = 500 µM in DMEM and DMEM+10% FBS, [**C1**] = 50 µM, all reactions performed at 37 °C, [**P1**]:[**C1**] = 1:5 (molar ratio); [**P1**] = ~ 10 µM (~ 1 mg/mL).

1.5.6 Investigation of side product formation

The most common side products during metal carbene reactions as reported in literature are diazo carbene dimer and OH insertion product. To elucidate if these side reactions occur, we did several control experiments. Hereto, diazo substrate **2a** was treated with catalyst **C4** using the same reaction conditions as described before in water. With the help of SIM (selective ion monitoring) channels, we looked specifically at the OH insertion product **4a** and dimer **5a** (Figure S17), as this allows high degree of sensitivity and precision. Even after 49 h of reaction, diazo substrate **2a** remained in the reaction mixture. However, small amounts of the OH insertion product **4a** and dimer **5a** were found (Figure S17B). As these peaks are not significantly visible during catalytic reactions performed, we ruled out their formation when using **P1@C1** as the catalyst.

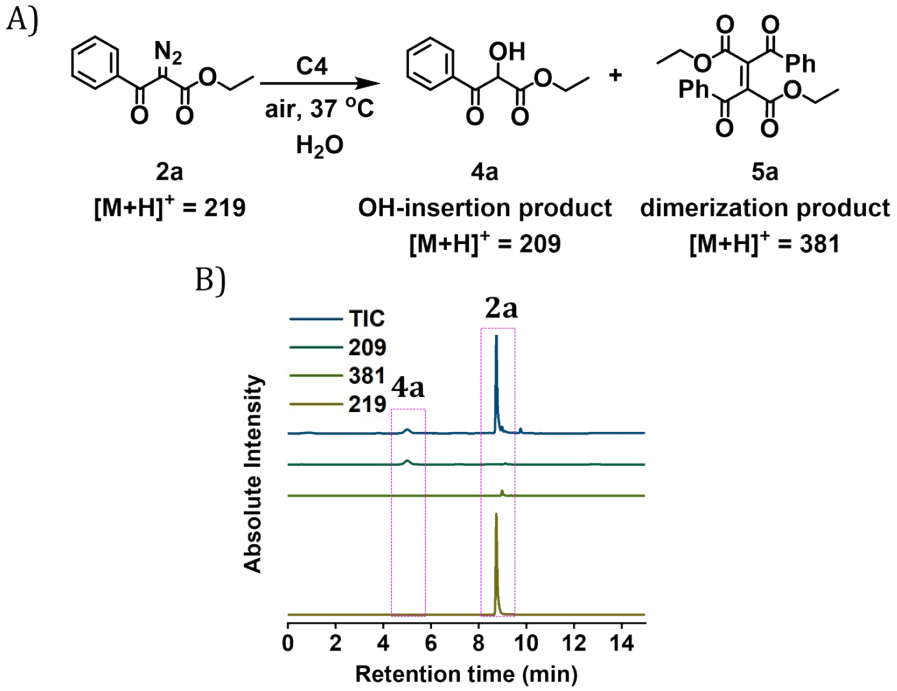


Figure S17: A) Formation of side products by OH insertion to product **4a** and dimerization to **5a** from **2a** in the presence of **C4**; B) HPLC-MS chromatogram of the reaction of **2a** in the presence of **C4** in water after 24 h. Each side product mass was added to SIM channel, reaction conditions [**2a**] = 500 µM in water [**C4**] = 50 µM, performed at 37 °C.

All reactions performed for evaluation of side products were performed in the presence of hydrophilic catalyst **C4**. In case of substrate **2b**, no major side products were observed as well. But in case of substrate **2c**, a significantly higher amount of dimer **5c** was formed while no OH insertion product **4c** was observed (Figure S18). The dimer formation could be due to the high reactivity of diazo substrate **2c** compared to **2a** and **2b**. **2a** and **2b** have two electron withdrawing substituents making them less reactive and less prone to decomposition when compared to **2c**, which only has one. But, when the reaction is catalyzed by **P1@C1**, only the peak corresponding to product **2c** was observed as major peak. This allowed us to conclude that the **P1@C1** catalyzed NH insertion reaction is highly selective towards the formation of the quinoxaline products and hence meet the criteria to be exported into biological environments.

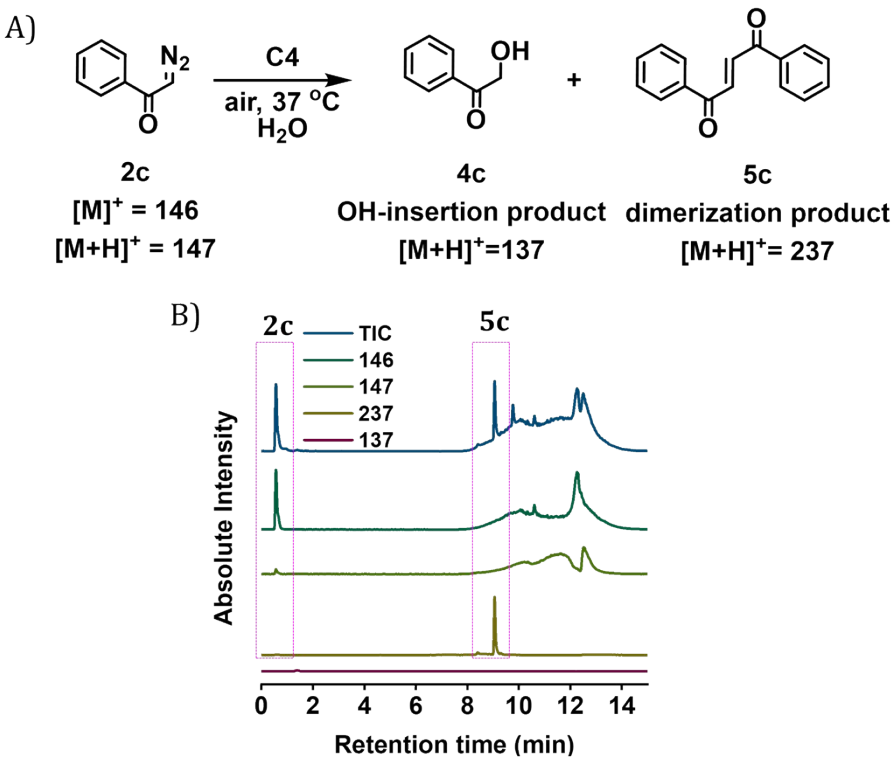


Figure S18: A) Formation of side products by OH insertion to product **4c** and dimerization to **5c** from **2c** in the presence of **C4**; B) HPLC-MS chromatogram of the reaction of **2c** in the presence of **C4** in water after 24 h. Each side product mass was added to SIM channel, reaction conditions [**2c**] = 500 µM in water [**C4**] = 50 µM, performed at 37 °C.

1.6 NH carbene insertion reactions in HeLa cells

1.6.1 Assessment of cell viability

HeLa cells were cultured and seeded in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and phenol red. Cytotoxicity of substrates, products and **P1@C1** was studied using the cell counting kit-8 (CCK-8) assay. HeLa cells were seeded in a 96-well plate. Wells were filled with 100 µL of cell suspension containing 8000 cells. The plate was then placed in an oven at 37 °C with 5% CO_2 flow for 24 hours. Then the compound to be studied was added to the cells. Substrates **1**, **2a**, and **2c** were added at concentrations of 25, 50, 100, 150 and 200 µM, **3a** at concentrations of 5, 10, 15, 25 and 50 µM and **3c** at concentrations of 5, 10, 15, 25, 50 and 100 µM. Compounds were added from freshly prepared stock solutions in DMSO. Stock solution concentration was chosen such that always 0.5 µL DMSO was always added. **P1@C1** was studied at concentrations of 5, 6, 7, 8, 9, 10, 11, 12, 13 and 15 µM. **P1@C1** stock solution (100 µM) was prepared in live cell imaging solution according to the procedure described above. The amount of stock solution volume to be added was first removed from the well to keep concentrations constant. Followingly, the plate was placed in the oven. After 24 hours, the medium was removed and 100 µL DMEM with 10% CCK8 were added to each well. The plate was then placed back in the oven at 37 °C for 2 to 4 hours. The absorbance of each well containing cells was measured at 450 nm at the microplate reader. Cell viability was determined as a fold change of the absorbance with respect to untreated cells.

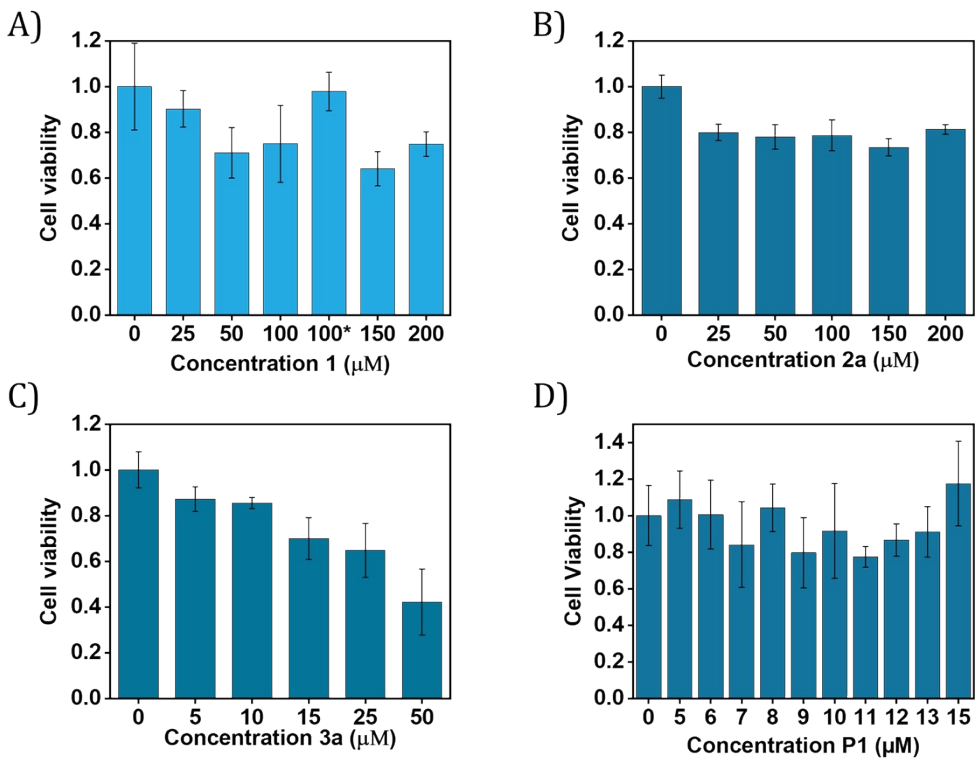


Figure S19: HeLa cells CCK8 cell viability assays when incubated with A) **1** (25 – 200 µM); B) **2a** (25 – 200 µM); C) **3a** (5 – 50 µM) and D) **P1@C1** (5 – 15 µM), all for 24 h. *Incubation time 14 h. The results are average of 3 wells.

*1.6.2 Procedure for NH carbene insertion reactions in HeLa cells for formation of **3a**:*
Cultured HeLa cells were seeded in a µ-Slide 18 well from Ibidi. Wells were filled with 100 µL of cell suspension containing 8000 cells. Cells were incubated with **P1@C1**, **1** and **2a** for the incubation time as mentioned. **P1@C1**, **1** and **2a** were mixed briefly together in the correct composition before addition to the cells. **P1@C1** was added from a freshly prepared stock solution (100 µM) in live cell imaging solution while **1** and **2a** were added from DMSO stock solutions (0.5 µL). After addition the µ-Slide was placed back in the oven. The cells were monitored in a confocal microscope.

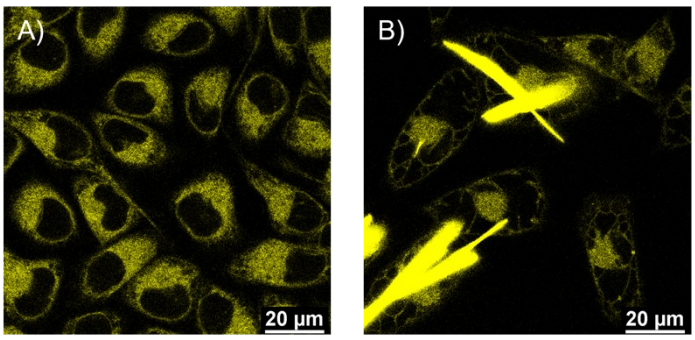


Figure S20: Confocal microscopy images of **3a** showcasing A) internalization in HeLa cells and B) crystallization. Cells were incubated with 20 µM **3a** (from stock solution in DMSO) for 24 h. λ_{exc} = 405 nm, λ_{em}= 411 – 580 nm. The formation of crystals outside the cells hints to extracellular product formation.

1.6.3 Quantification of fluorescent instensity of confcal microscopy images

Determination of mean gray values:

Image processing was performed in ImageJ using the grayscale images. Grayscale images were first binarized using the automatic thresholding in ImageJ. The binarized image was then divided over itself to create a mask in which the background is "not a number (NaN)". The original image was subsequently multiplied with this mask and the mean gray value was calculated. By making the background NaN instead of 0, the mean gray value of the cell area is calculated, canceling out differences in cell density between images. It should be noted here that the original image is used for the mask, which therefore excludes cells which have not taken up any fluorescent compound.

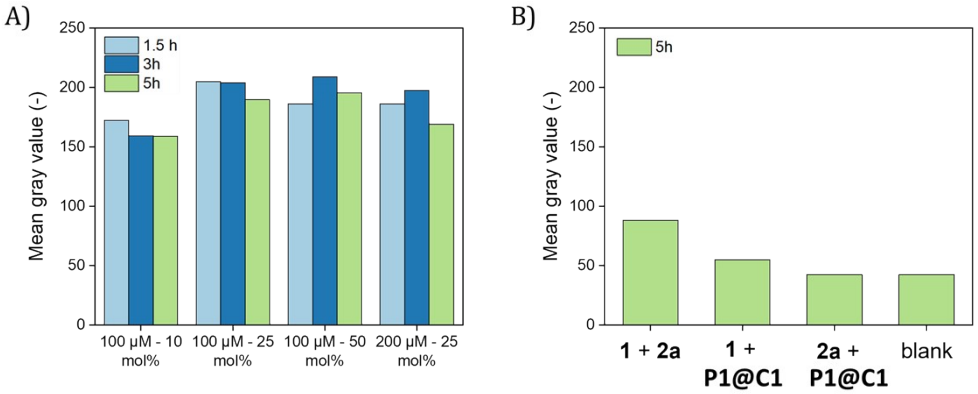


Figure S21: Quantification of fluorescence intensities of confocal microscopy images. A) Intensities obtained for catalytic experiments; B) Intensities obtained for control experiments with 100 µM **1** and **2a** and 2 µM **P1@C1** ([**C1**] = 10 µM). Cells were incubated with **1** and **2a** (from stock solution in DMSO) and 2 µM **P1@C1** ([**C1**] = 10 µM) (from stock solution in live cell imaging solution).

*1.6.4 Procedure for NH carbene insertion reactions in HeLa cells for formation of **3c**:*

The procedure was followed as described above for the formation of **3a.** After the indicated reaction times the medium was removed and 100 µL DMEM with 10% CCK8 were added to each well. The plate was then placed back in the oven at 37 °C for 2 to 4 hours. The absorbance of each well containing cells was measured at 450 nm at the microplate reader. Cell viability was determined as explained before.

*1.6.5 Control experiments for **3c** formation in HeLa cells:*

We performed control experiments with only **1**+**2c**, **1**+**P1@C1** and **2c**+**P1@C1** at 100 µM of substrates and 2 µM of **P1@C1** ([**C1**] = 10 µM). The combination **1**+**2c** and **1**+**P1@C1** when incubated with cells did not decrease the cell viability as expected. Unexpectedly, the combination **2c**+**P1@C1** induced cell death with a cell viability of only 14%, which is even less than what is observed for **3c** (Figure S21 and Figure 7B). We hypothesize that in the absence of diamine **1**, metal carbene radicals may have formed between **2c** and **C1** which induced the generation of harmful ROS (reactive oxygen species) triggering cell death.[4] As the cells incubated at the same concentration of **2c**+**P1@C1** in the presence of diamine **1** exhibited good cell viability, formation of these ROS during NH insertion reactions can be ruled out (Figure 7C).

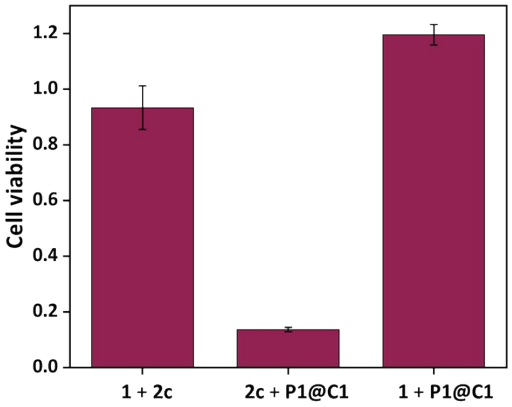


Figure S22: CCK-8 cell viability assays of control reactions in HeLa cells with **1**+**2c**, **2c**+**P1@C1** and **1**+**P1@C1**, [**1**] = [**2c**] = 100 µM, [**C1**] = 10 µM, [**P1**] = 2 µM, incubated 14 h. The results are the average of 3 wells.

1.7 References

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