

# Palladium-mediated intracellular chemistry



**Post-print of peer-reviewed article published by the Nature Publishing Group. Published article available at: http://dx.doi.org/10.1038/NCHEM.981**

**Cite as:**
**Yusop, R. M., Unciti-Broceta, A., Johansson, E. M. V., Sanchez-Martin, R. M., & Bradley, M. (2011). Palladium-mediated intracellular chemistry. *Nature Chemistry*, 3(3), 239-243.**

**Manuscript received: 23/09/2010; Accepted: 16/12/2010; Article published: 06/02/2011**

# Palladium-mediated intracellular chemistry**

Rahimi M. Yusop,[1,2] Asier Unciti-Broceta,[1,3] Emma M. V. Johansson,[1] Rosario M. Sánchez-Martín[1] and Mark Bradley[1,*]

[1]EaStCHEM, School of Chemistry, Joseph Black Building, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JJ, UK.

[2]School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor Darul Ehsan, Malaysia.

[3]Current affiliation: Edinburgh Cancer Research UK Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road, Edinburgh EH4 2XR, UK.

[*]Corresponding author; e-mail: mark.bradley@ed.ac.uk

[**]This work was supported financially by the Government ofMalaysia (R.M.Y.) and the Swiss National Science Foundation (E.M.V.J.). R.M.S.M. thanks the Royal Society for a Dorothy Hodgkin Fellowship. The authors thank D. Kelly for his help with the confocal microscopy studies.

**Supporting information:**
Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry

**Author contributions**
R.M.Y. synthesized materials, performed cell-based experiments and analysed the data. A.U.B. designed and supervised the research, analysed the data and co-wrote the paper. E.M.V.J. synthesized materials, performed experiments and analysed the data. R.M.S.M. designed and supervised the experiments and analysed the data. M.B. came up with the concept, designed the research, analysed the data and co-wrote the paper.

**Abstract**

Many important intracellular biochemical reactions are modulated by transition metals, typically in the form of metalloproteins. The ability to carry out selective transformations inside a cell would allow researchers to manipulate or interrogate innumerable biological processes. Here, we show that palladium nanoparticles trapped within polystyrene microspheres can enter cells and mediate a variety of Pd0-catalysed reactions, including allylcarbamate cleavage and Suzuki–Miyaura cross-coupling. The work provides the basis for the customization of heterogeneous unnatural catalysts as tools to carry out artificial chemistries within cells. Such in cellulo synthesis has potential for a plethora of applications ranging from cellular labelling to synthesis of modulators or inhibitors of cell function.

**Main text**

Cellular biochemistry is governed by a wide range of enzymatic entities, with the complex cellular machinery engineered to perform a myriad of diverse chemical reactions[1, 2]. Many of these reactions are catalysed by transition metals, typically in the form of metalloproteins[3-5], which modulate a wide variety of transformations, from highly selective oxidations to the efficient formation, lysis or isomerization of multifarious chemical bonds[1, 2]. In this context, many groups have taken advantage of the properties of these proteins with the generation of bio-inspired devices, based on coordination complexes aimed at mimicking biological function[6-11]. Bioorganometallic chemistry has therefore evolved as a fascinating field, full of possibilities and creative applications, ranging from highly selective chemistries to the manipulation and *in situ*interrogation of innumerable biological processes[12-16]. A fluorogenic chemosensor for the *in vivo* detection of $Pd^{2+}$ species has recently been described[17], but the use of $Pd^{0}$ catalysts to synthesize exogenous materials in cells has never been explored.

Of significant relevance is the work of Meggers, who described a water-soluble ruthenium complex that rapidly enters cells and performs an allylcarbamate cleavage from *bis*-*N*,*N*′-allyloxycarbonyl rhodamine 110 (**1**) (ref. 18), and proving to be non-toxic to cells during the short duration of the experiment. We reasoned that the use of a purely heterogeneous catalyst in the form of an ‘artificial organelle’ would allow the long-term cytoplasmic presence of metals with minimal leakage and toxicity (many transition metals, including palladium[19], trigger cell death[20]). This would then allow the exploration of challenging metal-catalysed reactions inside a cell.

**Results and discussion**

Encouraged by the vast possibilities and applications of palladium chemistry[21], the preparation of a bio-friendly internalizable $Pd^{0}$-based heterogeneous catalyst was undertaken. This was achieved through the application of two technologies. First, a microsphere (500 nm) mediated delivery system was used that had previously been applied in both cellular labelling and intracellular delivery of biomaterials, showing remarkable cellular biocompatibility and exonuclear localization[22-26]. The second technology involved the application of palladium nanoparticles entrapped within crosslinked resin beads, which have been shown to operate catalytically in a truly heterogeneous manner[27].

This innovative catalyst-loaded delivery system was synthesized as shown in Fig. 1. Amino-functionalized polystyrene microspheres (500 nm) were synthesized by dispersion polymerization from styrene-based monomers as previously described.[22] The electron-rich microsphere network binds $Pd^{2+}$ by coordination to the free amino groups and aromatic rings, while reduction of $Pd^{2+}$ to $Pd^0$ leads to aggregation and the generation of $Pd^0$nanoparticles (Fig. 1). Extensive crosslinking of the available amino groups on the particles means that the stable $Pd^0$ nanoparticles are permanently entangled and trapped within the microsphere. Finally, $Pd^0$ microspheres were fluorescently labelled to allow intracellular tracking of the catalysts (Supplementary Sections S2 and S3). Two different dyes were used for analytical reasons: Cy5.5-labelled $Pd^0$ microspheres were used for flow cytometry analyses and Texas Red-labelled $Pd^0$microspheres were used for the confocal studies.

Analysis of the resulting microspheres by transmission electron microscopy (TEM) showed palladium nanoparticles evenly distributed on the microsphere (Fig. 1b,c), with the presence of the $Pd^0$confirmed by powder X-ray diffraction (XRD) analysis (Fig. 1d). To demonstrate the catalytic activity of the microsphere-captured $Pd^0$ nanoparticles, the *bis*-*N*,*N*′-allyloxycarbonyl rhodamine 110 (**1**) used by Meggers[18] was incubated with $Pd^0$ microspheres under different conditions (Supplementary Tables S3–S5). The $Pd^0$ catalyst mediated the effective deprotection of the allyloxycarbonyl group, resulting in the liberation of the strongly fluorescent rhodamine 110 (**2**). Interestingly, the catalytic activity of the $Pd^0$ microspheres was clearly enhanced by the presence ofglutathione (5 mM), with turnover numbers up to 30 in the presence of cell extract and glutathione.

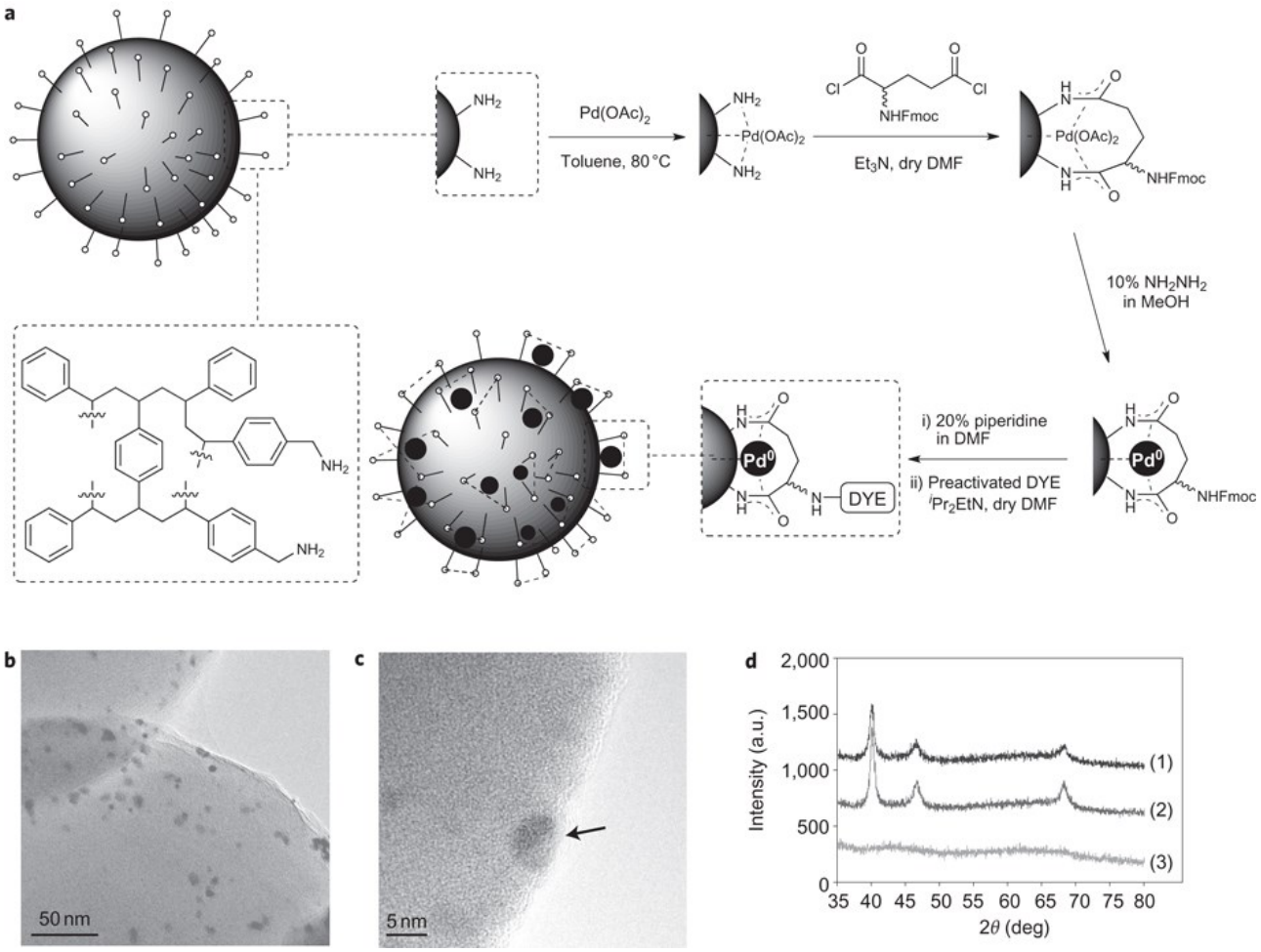

***Figure 1.*** **a**, Synthesis of fluorescently labelled $Pd^0$ microspheres. Amino-functionalized polystyrene microspheres[22] were treated with $Pd(OAc)_2$ for 3 h to ensure uptake and interaction with the beads. The coordinated $Pd^{2+}$ was subsequently trapped by extensive crosslinking with the *bis* acid chloride of racemic Fmoc-glutamic acid(generated *in situ*). Hydrazine in methanol was used to reduce $Pd^{2+}$ to $Pd^0$. Labelling of the $Pd^0$ microspheres was carried out by deprotection of the Fmoc group and subsequent treatment with an activated dye under basic conditions. **b**,**c**, TEM of $Pd^0$ nanoparticles within microspheres at two different magnifications: image of $Pd^0$ nanoparticles physically captured and entrapped on the microspheres (**b**); image of a single microsphere-captured $Pd^0$ nanoparticle (arrow, **c**). Average diameter of nanoparticles: 5 ± 2.5 nm. **d**, Powder XRD patterns of (1) $Pd^0$ microspheres, (2) commercial $Pd^0$ powder, and (3) naked microspheres.

Although the microspheres have shown remarkable cellular compatibility and have been widely used as a highly efficient cellular delivery vehicle for a variety of materials[22-26], the presence of $Pd^0$ on the beads required an investigation of their biological compatibility. $Pd^0$microspheres were therefore incubated with HeLa cells and uptake and toxicity evaluated. After 24 h, the intracellular presence of fluorescently labelled $Pd^0$ microspheres was determined by flow cytometry, demonstrating that more than 75% of cells had taken up one or more of the $Pd^0$microspheres, uptake that was further corroborated by confocal imaging (Supplementary Figs S6 and S7). Cytotoxicity associated with the $Pd^0$ microspheres, determined using both (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and propidium iodide assays, indicated low levels of necrosis (<4%) and good cell viability (>91%) after 48 h of contact with the $Pd^0$microspheres (Supplementary Figs S8 and S9, Tables S1 and S2).

*$Pd^0$-mediated allylcarbamate cleavage within cells*

The catalytic activity of the $Pd^0$ microspheres was investigated within living cells, using HeLa cells as a model system. Cells were loaded with fluorescently labelled $Pd^0$ microspheres, washed to eliminate extracellular $Pd^0$ microspheres, then fresh media containing 30 μM *bis*-*N*,*N*′-allyloxycarbonyl rhodamine 110 (**1**) was added. The lipophilic nature of non-fluorescent compound**1** allows its cellular internalization, whereas upon allylcarbamate cleavage most of the resulting fluorescent compound **2** is retained within the cell (Fig. 2a). As observed in Fig. 2, flow cytometry analysis of an untreated cell control (Fig. 2b), a control treated with only the Cy5.5-labelled $Pd^0$microspheres (Fig. 2c) or incubated just with reagent **1** (Fig. 2d) showed no fluorescence under the FITC emission filter (530/35 nm). In contrast, Cy5.5-labelled $Pd^0$ microsphere-loaded HeLa cells incubated with reagent **1** showed fluorescence emission under both Cy5.5 (indicating the presence of the $Pd^0$ microspheres) and FITC-like bandpass filters (associated with the deprotection of dye **2**) (Fig. 2e), showing $Pd^0$-mediated catalysis inside a cell for the first time. As shown in Fig. 2f, confocal microscopy verified the simultaneous presence of Texas Red-labelled $Pd^0$ microspheres and intracellular compound **2**.

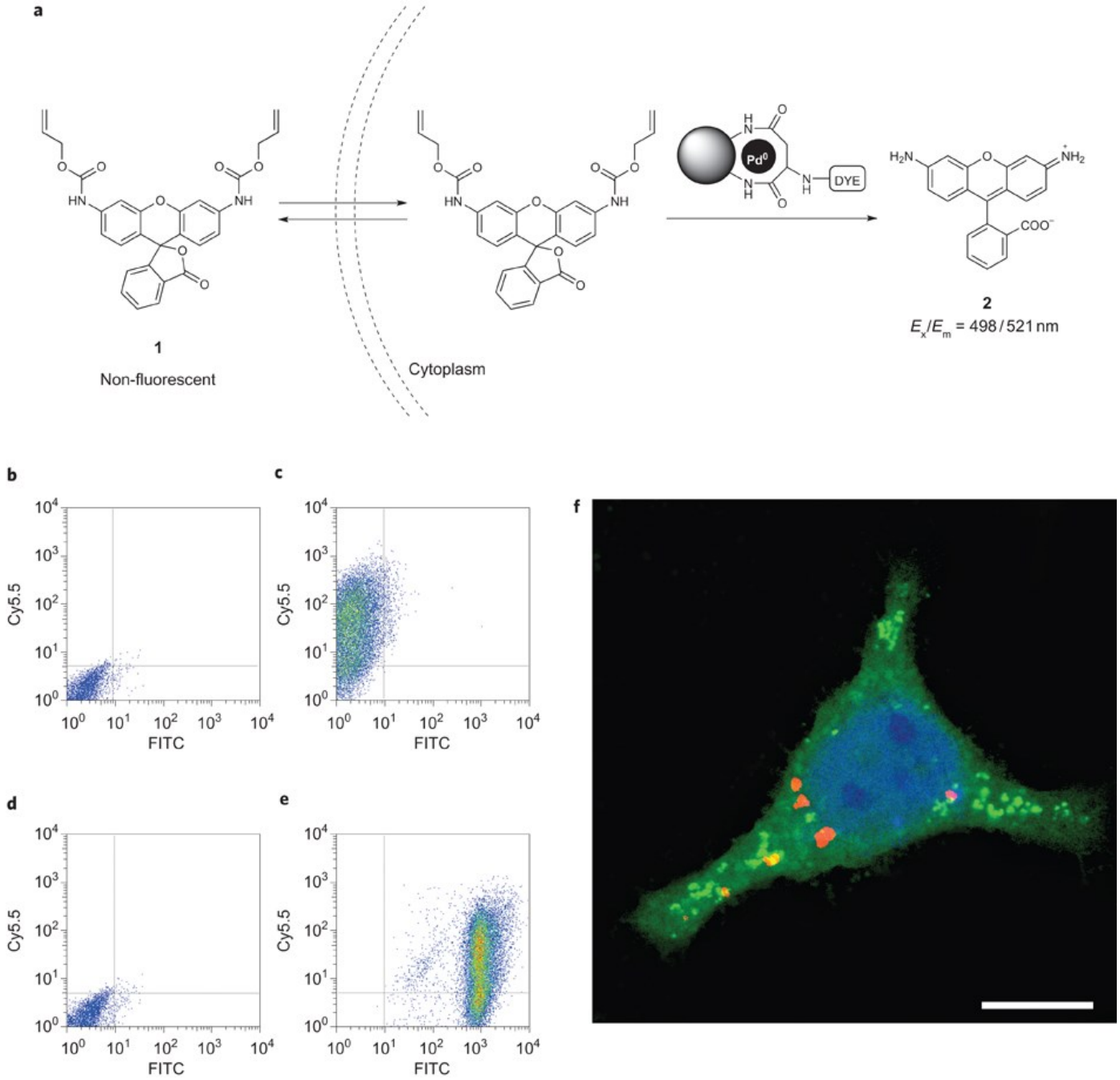


***Figure 2.*** **a**, $Pd^0$-catalysed intracellular deprotection of reagent **1** generates fluorescent compound **2**. **b**–**e**, Flow cytometry analysis of HeLa cells showing intracellular $Pd^0$catalysis of allylcarbamate cleavage. The *y*-axis represents Cy5.5 fluorescence intensity due to the Cy5.5-labelled $Pd^0$ microspheres (bandpass emission filter, 780/60 nm) and the *x*-axis the FITC-like intensity of the cell due to compound **2** (bandpass emission filter, 530/30 nm). **b**, Untreated cell control (no fluorescence emission). **c**, Cells after 24 h incubation with Cy5.5-labelled $Pd^0$ microspheres (positive fluorescence emission from Cy5.5 channel). **d**, Cells after 24 h incubation with reagent **1** (no fluorescence emission). **e**, Cy5.5-labelled $Pd^0$ microsphere-loaded cells after 24 h incubation with reagent **1**, showing fluorescence emission under both Cy5.5 (indicating the presence of the $Pd^0$ microspheres) and FITC-like bandpass filters (confirming the synthesis of deprotected dye **2**). **f**, Merged confocal image of a single HeLa cell (fixed with paraformaldehyde) showing a Hoechst 33342-stained nucleus (blue) with Texas Red-labelled $Pd^0$ microspheres (red) and deprotected compound **2** (green). Scale bar, 10 μm.

To expand the scope of the *in cellulo* deprotection method, a preliminary cytotoxicity study based on an allylcarbamate-protected derivative of amsacrine (a commercially available antineoplastic agent[28]) was performed (Supplementary Fig. S22). Alloc protection led to a measurable reduction in the cytotoxic properties of this chemically modified derivative relative to free amsacrine, a fact that was used to investigate if amsacrine could be released *in situ* by the catalytic activity of cell-containing $Pd^0$ microspheres. Cells incubated with alloc-protected amsacrine showed up to a sevenfold increment of cytotoxicity in cell cultures preloaded with $Pd^0$ microspheres.

*Making C–C bonds within cells*

The Suzuki–Miyaura cross-coupling of arylboronates or esters with aryl halides (or triflates) in the presence of $Pd^0$ permits ready access to a spectacular range of biaryls[29, 30]. To explore the intracellular formation of a carbon–carbon cross-coupled product, an unambiguous fluorescence-detectable cell-based experiment was designed, based on the palladium-mediated synthesis of a fluorescent dye (anthofluorescein[31]) via an aryl–aryl cross-coupling reaction (Fig. 3a). The lipophilic non-fluorescent mono-triflate **3**, containing a fluoran-based pro-fluorophore, and the alkylaminophenylboronate **4** were therefore synthesized. Cross-coupling of these two components would restore the π-electron conjugation of the fluoran polycycle **3** by opening of the lactone, resulting in molecular fluorescence while at the same time localizing the product **5** to the mitochondria due to the presence of a triphenylphosphonium moiety (lipophilic cations direct accumulation of several hundredfold within mitochondria[32]).

Suzuki–Miyaura dye *in cellulo* synthesis was initiated by the incubation of fluorescently labelled $Pd^0$microspheres with HeLa cells for 24 h, followed by intensive washing of the cells to eliminate extracellular $Pd^0$ microspheres before the addition of the two reagents **3** (20 μM) and **4** (20 μM). After 48 h incubation, cellular fluorescence was investigated by confocal microscopy with mitochondria labelled with a far-red dye (MitoTracker Deep Red, $E_x/E_m \approx$ 640/662 nm). Fixed cells were labelled with Hoechst 33342 (nuclei stain), imaged by confocal microscopy and the images processed for three-dimensional analysis. As shown in Fig. 3b, confocal images showed co-localization of both $Pd^0$-synthesized dye and MitoTracker, demonstrating the unambiguous identification of the Suzuki–Miyaura product **5**, with *in cellulo* synthesis confirmed by flow cytometry analysis (Supplementary Fig. S15). Controls were carried out, both in solution and in cell experiments, to rule out the possibility of any fluorescence being the result of hydrolysed mono-triflate **3** (Supplementary Figs S15, S19 and S20, Table S5). Indeed, mono-triflate was remarkably robust, surviving unchanged in the presence of $Pd^0$ for over 48 h. Compound **5** was also extracted from the cells and its identity confirmed by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) studies, confirming intracellular synthesis. Owing to the broad excitation/emission spectra of Texas Red, both the Suzuki–Miyaura product **5** and the Texas Red-labelled $Pd^0$ microspheres were imaged under the same fluorescent channel. The $Pd^0$microspheres could be identified, because they did not co-localize with the mitochondria tracker dye or the nucleus stain, thus corroborating their cytoplasmatic location (Fig. 3c; Supplementary Figs S16 and S17). This experiment represents the first non-enzymatic aryl–aryl bond formation ever achieved within living cells.

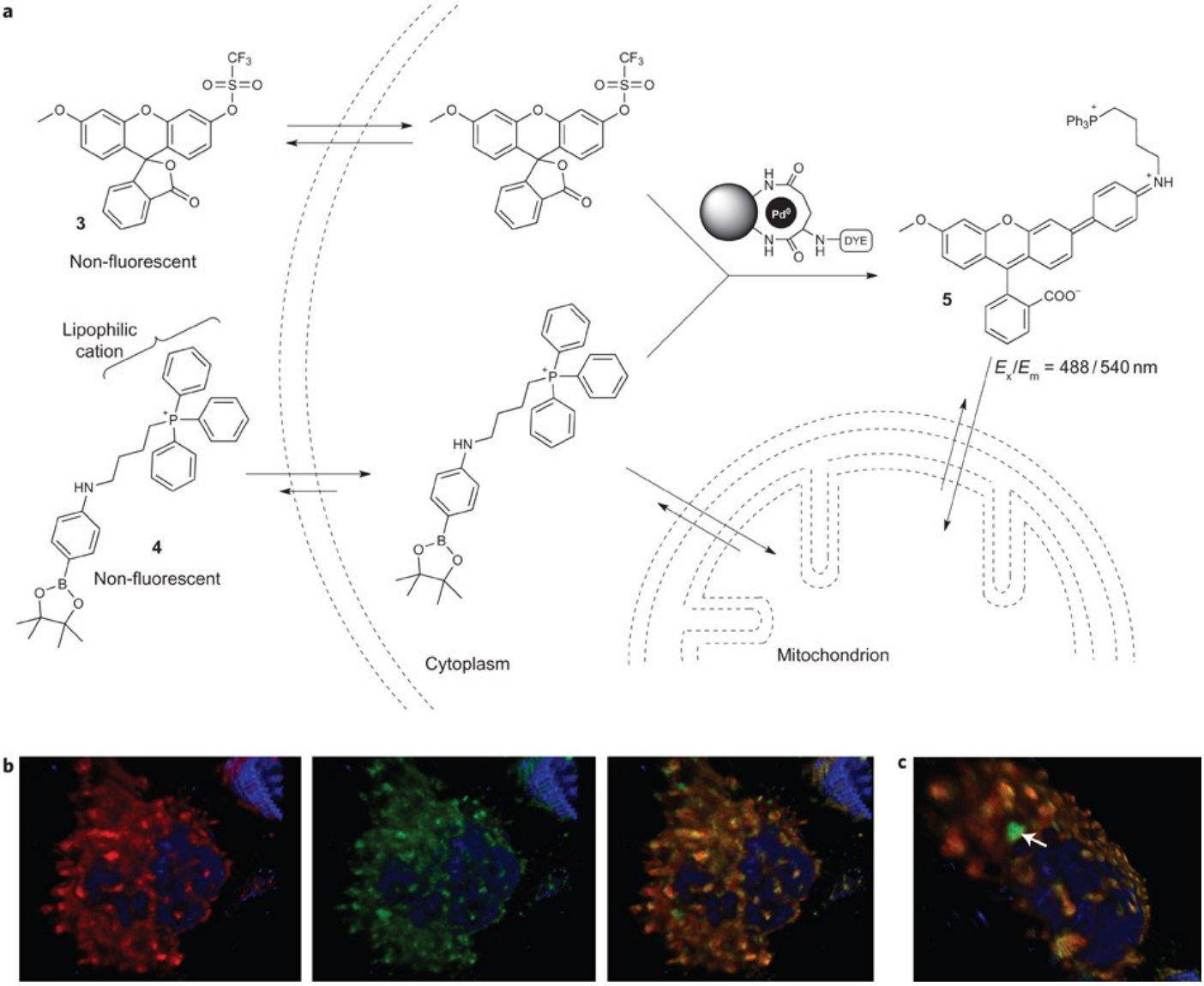


***Figure 3.*** **a**, $Pd^0$-catalysed intracellular cross-coupling of reagents **3** and **4** generates the mitochondria-localized fluorescent compound **5**. **b**,**c**, HeLa cells were loaded with $Pd^0$ microspheres, washed to eliminate extracellular $Pd^0$ microspheres, and subsequently incubated for 48 h with reagents **3** and **4**. Cells were incubated for 30 min with MitoTracker Deep Red (mitochondrial stain), fixed with paraformaldehyde, incubated with Hoechst 33342 (nuclei stain) and imaged by confocal microscopy. Deconvolved confocal images (**b**) of a single cell showing co-localization of MitoTracker-labelled mitochondria and Suzuki–Miyaura product **5**. Left panel: cell nucleus (blue) and mitochondria (red). Centre panel: cell nucleus (blue) and *in cellulo* synthesized compound **5** (green). Right panel: merged image (orange indicates co-localization). Merged image (**c**) of the same cell observed from a different angle. White arrow indicates the presence of a Texas Red-labelled $Pd^0$microsphere (not localized within the mitochondria), which was imaged using a 550/20 nm emission filter together with compound **5**.

In conclusion, the first $Pd^0$-based heterogeneous catalyst with the ability to cross cell membranes, stay harmlessly within the cytoplasm for days, and carry out artificial chemistry has been described. This engineered pseudo-organelle demonstrated intracellular catalytic activity towards exogenous materials, allowing chemistry never before achieved

within cells to be performed, such as the Suzuki–Miyaura cross-coupling reaction. This investigation provides the basis for the customization of heterogeneous unnatural catalysts as tools for creative applications in chemical biology (such as*in situ* labelling of cellular structures), pharmacology (for example, *in cellulo* pro-drug activation of hydrophilic molecules with low cell penetrability for functional screening in cellular disease models) and, potentially, in medicine (for example, the systemic administration of a pro-drug with local activation via implant-captured catalysts).

**Methods**

***$Pd^0$ microsphere synthesis:*** Aminomethyl polystyrene microspheres were prepared as previously described[22]. Microspheres (1 ml, aminomethyl polystyrene microsphere, 0.5 μm, 0.08 mmol $g^{-1}$, 4% solid content (SC)) were placed in a 1 ml eppendorf tube and the water removed after centrifugation at 13,000 r.p.m. for 5 min. The beads were subsequently washed with toluene (2 × 1 ml). A volume of 0.2 ml of a 0.4 mg $ml^{-1}$ palladium acetate solution in toluene was added and heated to 80 °C and sonicated every 2 min (5×). Subsequently, the reaction mixture was shaken at room temperature for 2 h to give a light-brown coloured mixture. The bead mixture was washed with toluene (3 × 1 ml) to ensure excess of palladium acetate was removed. Cross-coupling reagent (10 mg, Fmoc-Glu(Cl)-Cl) was freshly prepared and dissolved in 1 ml of dry dimethylformamide (DMF) together with triethylamine(11 μl, 0.08 mmol). Subsequently, 0.5 ml of this coupling solution was added to the beads and shaken for 1 h at room temperature to afford 100% crosslinked beads. The resulting microspheres were washed with methanol (3 × 0.5 ml) and water (3 × 0.5 ml). The $Pd^0$ microspheres were stored in water as a light-grey suspension. The Fmoc group was deprotected using 20% piperidine inDMF, and the $Pd^0$ microspheres were washed with DMF (3 × 1 ml), then treated with pre-activated dye (5 equiv. Cy5.5 or Texas Red, 0.5 mg $ml^{-1}$ in DMF) and diisopropylethylamine (DIPEA) (10 equiv.). The resulting mixture was shaken for 24 h at room temperature. The fluorescently labelled $Pd^0$ microspheres were washed with MeOH (3 × 1 ml) and water (3 × 1 ml) and stored in water in the dark. Cy5.5-labelled $Pd^0$ microspheres were used for the flow cytometry analysis, and Texas Red-labelled $Pd^0$ microspheres were used for the confocal microscopy studies.

***$Pd^0$-mediated allylcarbamate cleavage in HeLa cells:*** For flow cytometry analysis, HeLa cells were plated in Roswell Park Memorial Institute (RPMI) supplemented with serum and antibiotics (RPMI Complete Media (RPMI-CM)) in a 12-well plate with a density of 40,000 cells per well and the cells were grown for 24 h. Thereafter, 1.0 μl of $Pd^0$microspheres per ml (Cy5.5 labelled for flow cytometry analysis and Texas Red labelled for confocal analysis) were added and incubated with the cells for 24 h. Excess $Pd^0$ microspheres were removed by washing with phosphate buffered saline (PBS) (3×). Protected Rhodamine 110 (**1**) was added (30 μM in RPMI-CM) and incubated with the cells at 37 °C and in 5% $CO_2$ for 24 h. After incubation, cells were washed twice with PBS, harvested with trypsin/ethylenediaminetetraacetic acid (EDTA), washed again with PBS and resuspended in 2% fetal calf serum (FCS) in PBS buffer. Cell fluorescence was analysed by flow cytometry using a FACS Aria flow cytometer (Becton Dickinson). A total of 10,000 events per sample were analysed. A 530/30 nm bandpass filter (FITC) was used for

Rhodamine 110 (**2**) and 780/60 nm for Cy5.5 detection. For the confocal microscopy study, HeLa cells were cultured on sterilized circular glass slide cover slips (24 mm), which were coated with 0.01% polylysine in water for 5 min at room temperature. The cover slips were washed with PBS (3×) before use. The glass cover slips were placed in six-well plates with 90,000 cells per well and incubated overnight following the procedure described above. HeLa cells were then fixed with 4% formaldehyde in PBS for 30 min at room temperature. Nuclei were stained by incubation with a 10 μg $ml^{-1}$ solution of Hoeschst 33342 in media for 5 min at 37 °C before analysis. Microscope settings: excitation laser lines at 488, 543 and 595 nm, with emission filters of 385–470 nm for Hoechst 33342, 505–530 nm for Rhodamine 110 and 595–615 nm for Texas Red.

***$Pd^0$-mediated Suzuki cross-coupling in HeLa cells:*** HeLa cells were plated on sterilized glass cover slips (24 mm) placed in a six-well plate (90,000 cells per well) and incubated at 37 °C for 24 h. Media were removed and replaced with fresh media containing Texas Red-labelled $Pd^0$ microspheres (2 μl, $1.52 \times 10^{10}$ beads per well) and incubated at 37 °C for 24 h. Excess of extracellular $Pd^0$ microspheres was removed by washing with PBS (3×). Compounds **3** and **4** (20 mM in dimethyl sulfoxide (DMSO)) were diluted in fresh media to give a final concentration of 20 μM and incubated at 37 °C and in 5% $CO_2$ for 48 h. Subsequently, mitochondria were stained by 30 min incubation with 50 nM of MitoTracker Deep Red in RPMI-CM at 37 °C. HeLa cells were then fixed with 4% formaldehyde in PBS (30 min at room temperature), and nuclei were stained by the addition of a 10 μg $ml^{-1}$ solution of Hoechst 33342 (5 min). Microscope settings: excitation laser lines at 488 nm, 595 nm and 633 nm, with emission filters of 385–470 nm for Hoechst 33342, 540–560 nm for compound **5** and 650–670 nm for MitoTracker Deep Red.

**References**


[1] Silverman, R. E. *Organic Chemistry of Enzyme-Catalyzed Reactions* 2nd edn (Academic Press, 2002).

[2] Bugg, T. *Introduction to Enzyme and Coenzyme Chemistry* 2nd edn (Wiley-Blackwell, 2004).

[3] Lippard, S. J. & Berg, J. M. *Principles of Bioinorganic Chemistry* (University Science Books,1994).

[4] Andreini, C., Bertini, I., Cavallaro, G., Holliday, G. L. & Thornton, J. M. Metal ions in biological catalysis: from enzyme databases to general principles. *J. Biol. Inorg. Chem.* **13**, 1205–1218(2008).

[5] Waldron, K. J., Rutherford, J. C., Ford, D. & Robinson, N. J. Metalloproteins and metal sensing. *Nature* **460**, 823–830 (2009).

[6] Costas, M., Mehn, M. P., Jensen, M. P. & Que, L Jr. Dioxygen activation at mononuclear nonheme iron active sites: enzymes, models, and intermediates. *Chem. Rev.* **104**, 939–986(2004).

[7] Tshuva, E. Y. & Lippard, S. J. Synthetic models for non-heme carboxylate-bridged diiron metalloproteins: strategies and tactics. *Chem. Rev.* **104**, 987–1012 (2004).

[8] Lippard, S. J. The inorganic side of chemical biology. *Nat. Chem. Biol.* **2**, 504–507 (2006).

[9] Tolman, W. B. & Spencer, D. J. E. New advances in ligand design for synthetic modeling of metalloprotein active sites. *Curr. Opin. Chem. Biol.* **5**, 188–195 (2001).

[10] Xing, G. & DeRose, V. J. Designing metal–peptide models for protein structure and function.*Curr. Opin. Chem. Biol.* **5**, 196–200 (2001).

[11] Baltzer, L. & Nilsson, J. Emerging principles of *de novo* catalyst design. *Curr. Opin. Biotech.***12**, 355–360 (2001).

[12] Lu, Y. Biosynthetic inorganic chemistry. *Angew. Chem. Int. Ed.* **45**, 5588–5601 (2006).

[13] Haas, K. L. & Franz, K. J. Application of metal coordination chemistry to explore and manipulate cell biology. *Chem. Rev.* **109**, 4921–4960 (2009).

[14] Domaille, D. W., Que, E. L. & Chang, C. J. Synthetic fluorescent sensors for studying the cell biology of metals. *Nat. Chem. Biol.* **4**, 168–175 (2008).

[15] McRae, R., Bagchi, P., Sumalekshmy, S. & Fahrni, C. J. *In situ* imaging of metals in cells and tissues. *Chem. Rev.* **109**, 4780–4827 (2009).

[16] Liang, G., Ren, H. & Rao, J. A biocompatible condensation reaction for controlled assembly of nanostructures in living cells. *Nature Chem.* **2**, 54–60 (2010).

[17] Santra, M., Ko, S.-K., Shin, I. & Ahn, K. H.Fluorescent detection of palladium species with an*O*-propargylated fluorescein. *Chem. Commun.* **46**, 3964–3966 (2010).

[18] Streu, C. & Meggers, E. Ruthenium-induced allylcarbamate cleavage in living cells. *Angew. Chem. Int. Ed.* **45**, 5645–5648 (2006).

[19] Bruijnincx, P. C. A. & Sadler, P. J. New trends for metal complexes with anticancer activity.*Curr. Opin. Chem. Biol.* **12**, 197–206 (2008).

[20] Abu-Surrah, A. S. & Kettunen, M. Platinum group antitumor chemistry: design and development of new anticancer drugs complementary to cisplatin. *Curr. Med. Chem.* **13**,1337–1357 (2006).

[21] Negishi, E. I. *Handbook of Organopalladium Chemistry for Organic Synthesis* (Wiley-Interscience, 2002).

[22] Sanchez-Martin, R. M. *et al*. Bead-based cellular analysis, sorting and multiplexing.*ChemBioChem* **6**, 1341–1345 (2005).

[23] Sanchez-Martin, R. M., Cuttle, M., Mittoo, S. & Bradley, M. Microsphere-based real-time calcium sensing. *Angew. Chem. Int. Ed.* **45**, 5472–5474 (2006).

[24] Alexander, L. M., Sanchez-Martin, R. M. & Bradley M. Knocking (anti)-sense into cells: the microsphere approach to gene silencing. *Bioconjug. Chem.* **20**, 422–426 (2009).

[25] Sanchez-Martin, R. M. *et al*. Microsphere-mediated protein delivery into cells. *ChemBioChem***10**, 1453–1456 (2009).

[26] Alexander, L. M. *et al*. Investigation of microsphere-mediated cellular delivery by chemical, microscopic and gene expression analysis. *Mol. BioSyst.* **6**, 399–409 (2010).

[27] Cho, J. K. *et al*. Captured and cross-linked palladium nanoparticles. *J. Am. Chem. Soc.* **128**,6276–6277 (2006).

[28] Horstmann, M. A. *et al*. Amsacrine combined with etoposide and high-dose methylprednisolone as salvage therapy in acute lymphoblastic leukemia in children.*Haematologica* **95**, 1701–1703 (2005).

[29] Miyaura, N. & Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **95**, 2457–2483 (1995).

[30] Alonso, F., Beletskaya, I. P. & Yus, M. Non-conventional methodologies for transition-metal catalysed carbonecarbon coupling: a critical overview. Part 2: The Suzuki reaction.*Tetrahedron* **64**, 3047–3101 (2008).

[31] Unciti-Broceta, A., Yusop, M. R., Richardson, P., Walton, J. & Bradley, M. A fluorescein-derived anthocyanidin-inspired pH sensor. *Tetrahedron Lett.* **50**, 3713–3715 (2009).

[32] Smith, R. A., Porteous, C. M., Gane, A. M. & Murphy, M. P. Delivery of bioactive molecules to mitochondria *in vivo*. *Proc. Natl Acad. Sci. USA* **100**, 5407–5412 (2003).