Innovative Therapeutics Lab

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EDI in THERACAT

www.boomchemistry.com

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THE UNIVERSITY OF EDINBURGH

CANCER RESEARCH UK

IGMM

*Strategic partnership of the **Medical Research Council Human Genetics Unit**, the **Cancer Research UK Edinburgh Centre** and the **Centre for Genomic & Experimental Medicine**.*

The IGMM partnership brings together engineers, scientists and clinicians from fields as diverse as mathematics, computer sciences, bioinformatics, chemistry, biology and medicine to study human health and disease using modern multidisciplinary approaches. Over 70 PIs and 500 staff and PhD students are working together in a single, scientific endeavour.

Innovative Therapeutics Lab

Expertise

From the beaker to the theatre

Chemistry
Prodrugs

Surgery
Glioma

Nanomaterials
Analysis

Prostate cancer models

Phenotypic assays

zebrafish models

Glioma models

Funders

EPSRC

igmm
INSTITUTE OF GENETICS & MOLECULAR MEDICINE

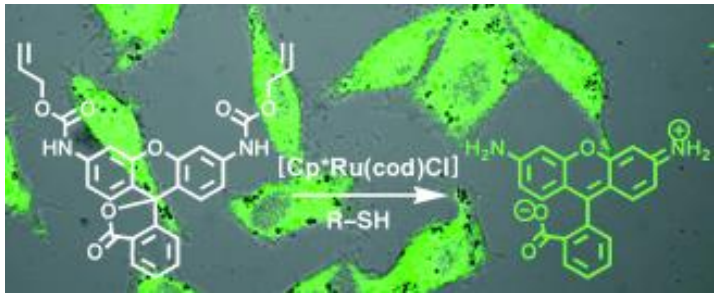
MRC Medical Research Council

THE UNIVERSITY OF EDINBURGH

CANCER RESEARCH UK

European Commission

Non-Biological Metal Catalysis in Cells



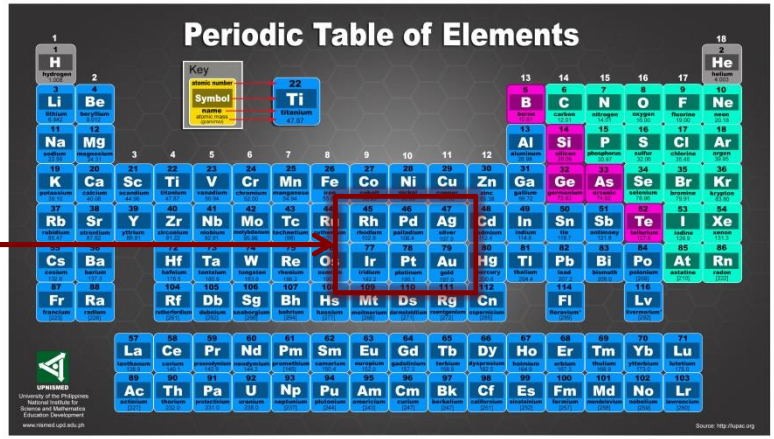
Streu & Meggers, *Angew. Chemie* **2006**, *45*, 5645

Antecedents: *Meggers developed a water-soluble ruthenium complex that rapidly entered cells and performed an allylcarbamate cleavage, while proving to be non-toxic to cells during the short duration of the experiment (minutes).*

CHALLENGE: *Reactions mediated by non-biological transition metals in living systems.* ***REQUIREMENT:*** *Elimination / control of the inherent toxicity of the metal.*

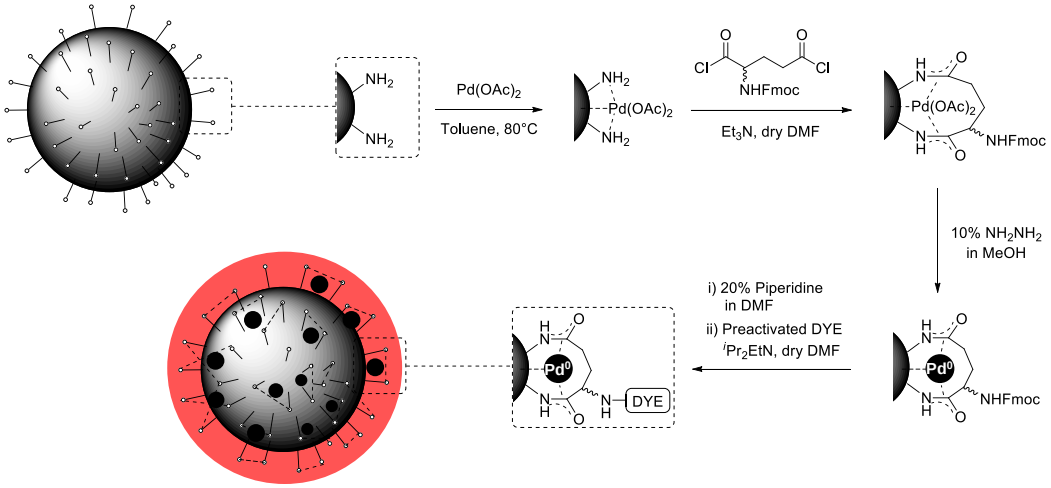
Toxicity & Mechanism:
Metals from the platinum group trigger cell death by cross-linking of DNA >>
HIGHLY TOXIC

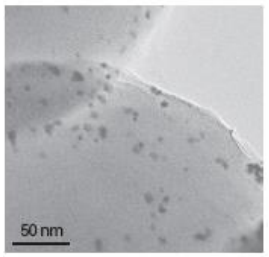
Hypothesis:
Restriction of the catalyst's freedom to enter cell nuclei will suppress its toxicity mechanism >>
HETEROGENEOUS CATALYST

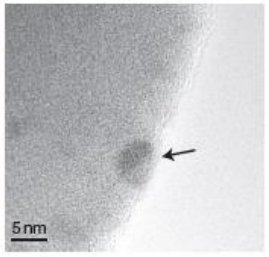


Palladium Chemistry in Cells

A "bio-friendly" cell-penetrating Pd^0 heterogeneous catalyst

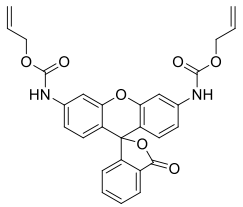






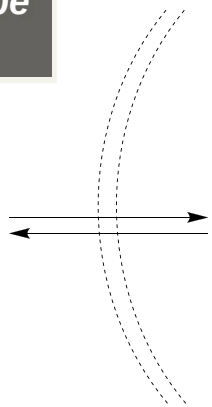
Biocompatible microspheres that penetrate cells and stay in the cytoplasm >> exonuclear location >> minimal toxicity

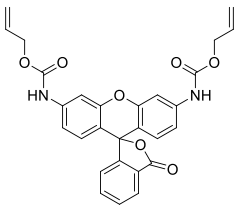
Pd^0 Sensitive Probe (lipophilic)



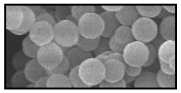
1

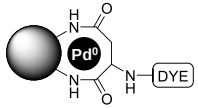
Non-Fluorescent



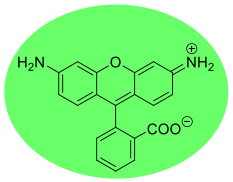


Cytoplasm





Active Dye (hydrophilic)



2

Ex / Em = 498 / 521 nm

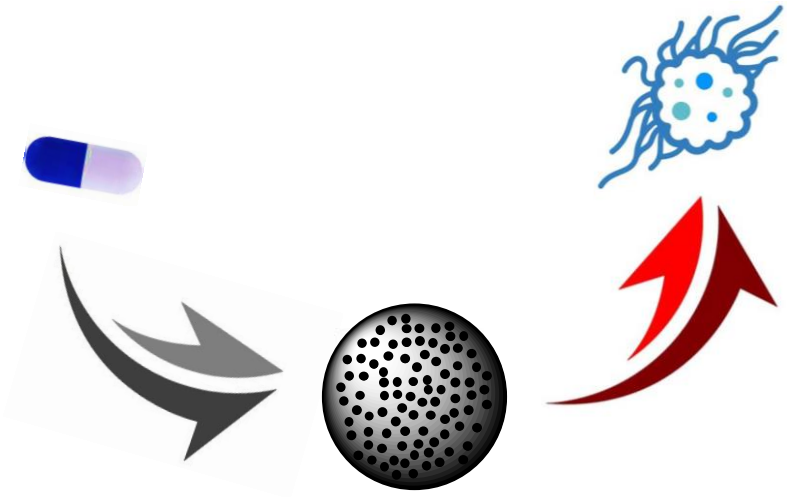
Nat Chemistry **2011** *3*, 241–245

Nat Protocols **2012** *7*, 1207-1212

SAFE ON THEIR OWN,

CYTOTOXIC TOGETHER

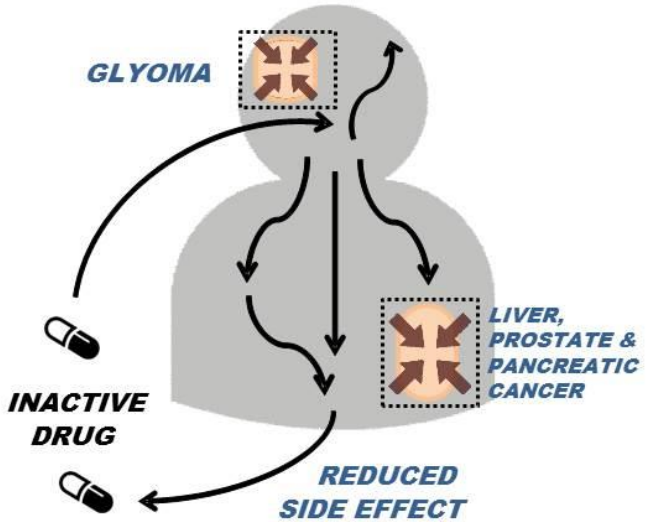
Concept

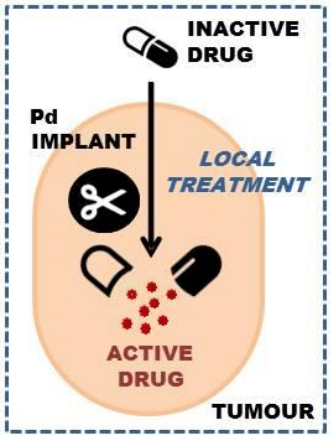


The prodrug is inactive until it meets its activating device:

- → ***Systemic administration***
- → ***Local activation***
- → ***The activating device is not an enzyme but an artificial device functionalized with a metal catalyst***

A NEW PRODRUG STRATEGY



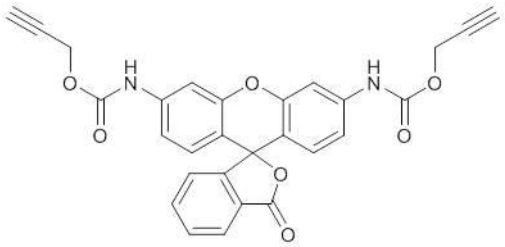


> ***Site specific drug release***

> ***Compatible with multidose regimes***

- ***BIOORTHOGONAL PRODRUG:*** *inactive drug exclusively activated by* ***BOOM chemistry***
- ***PALLADIUM IMPLANT:*** *safe device that converts the* ***prodrug*** *into the* ***active drug***

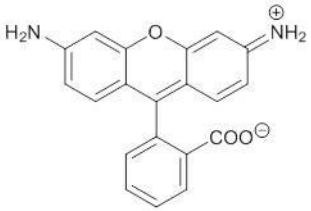
In vivo LOCAL Activation of PROBE



Pd-Labile PROBE

Non Fluorescent / Lipophilic

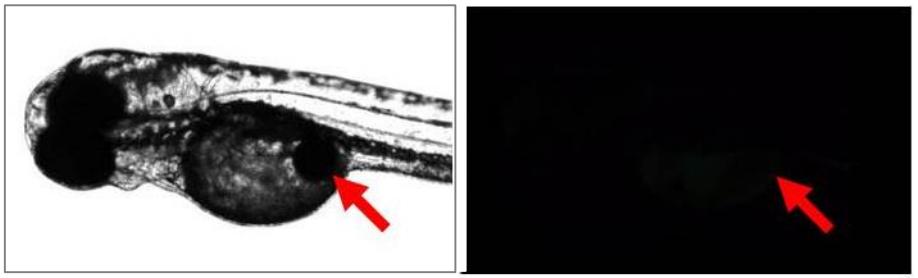
Pd^0-resin (inside yolk of zebrafish embryo)



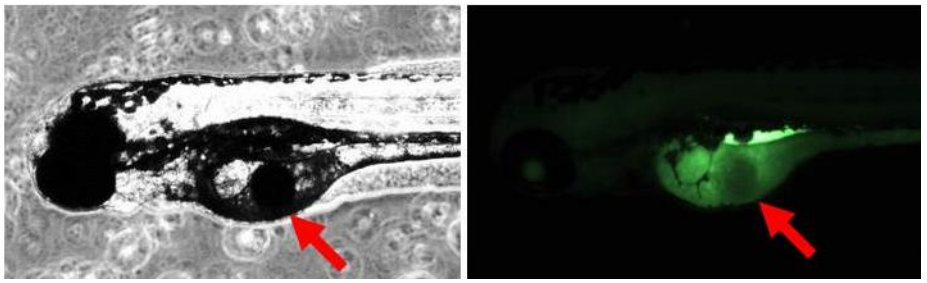
Rhodamine 110

Fluorescent / Hydrophilic

Pd-resin



Pd^0-resin + PROBE

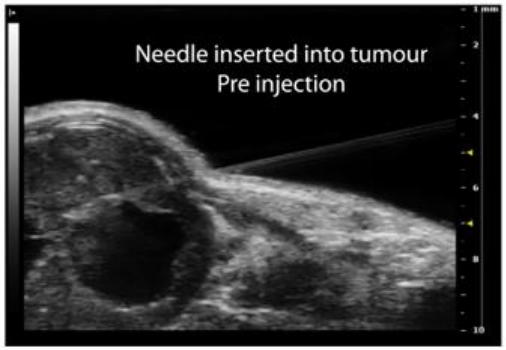


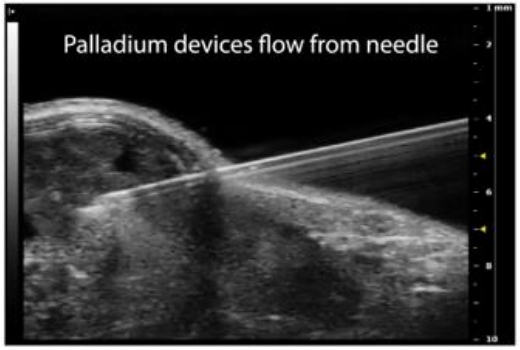
Strong fluorescent signal was clearly observed from the area surrounding the Pd^0-resin in the yolk sac, confirming that the palladium-functionalized device is catalytically active in vivo

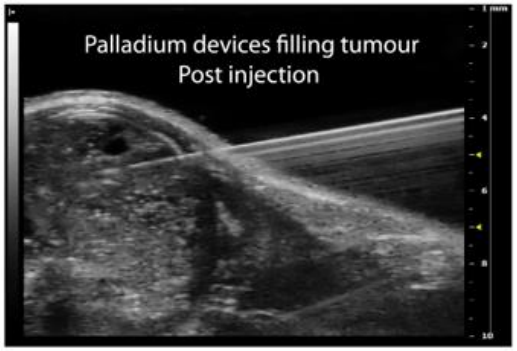
Nat Commun **2014**, 5, 3277

Validation of the catalyst insertion

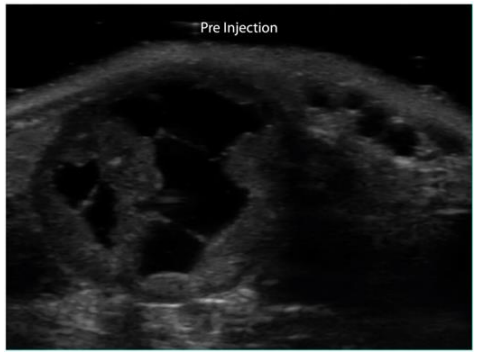
Beads are echogenic: the insertion in the tumor is guided by Ultrasound

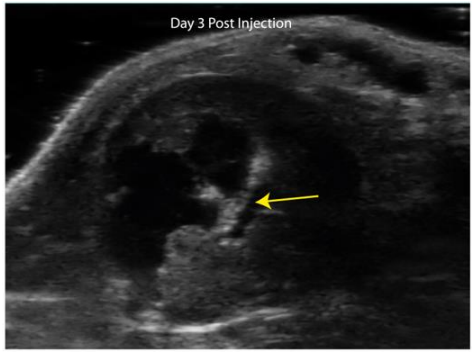


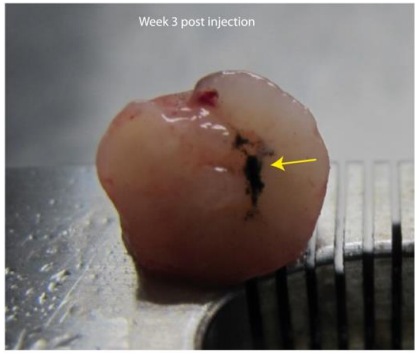




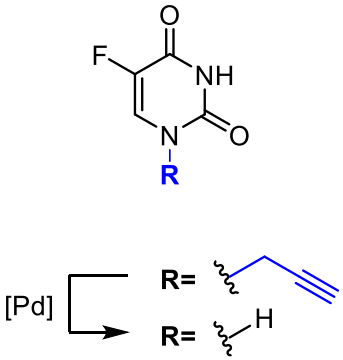
Ultrasound guided intratumoural injection of Pd-devices in a xenograft tumour in mice



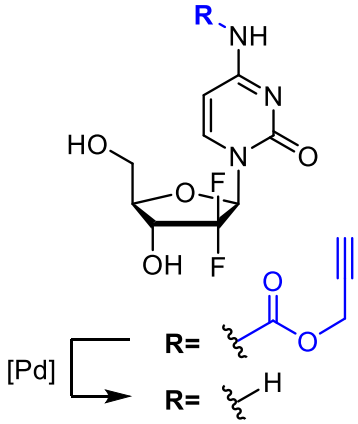




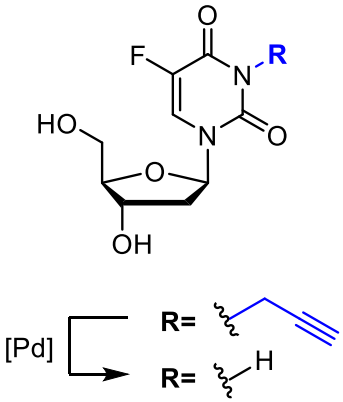
Pd-Activated Prodrugs



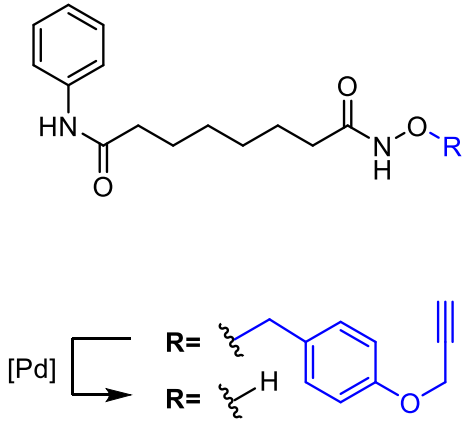
Nat. Commun. **2014**, *5*, 3277



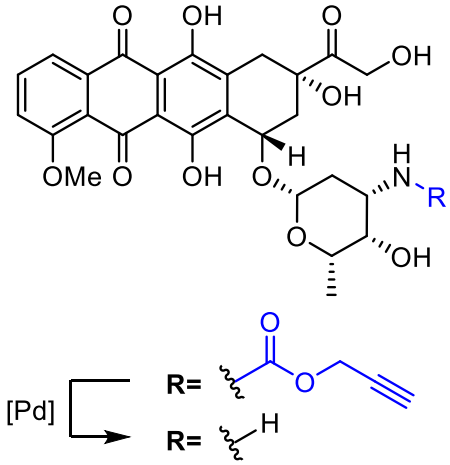
J. Med. Chem. **2014**, *57*, 5395



Sci. Rep. **2015**, *5*, 9329



J. Med. Chem. **2016**, *59*, 9974

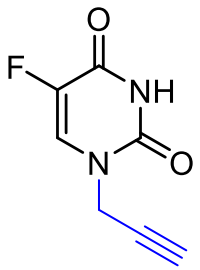


Angew. Chemie **2017**, *56*, 12548

Prodrug Safety and Activation

Alkylation of the N1 position of 5FU (cytotoxic drug used to treat colorectal and pancreatic cancer) resulted in biochemically-stable inactive derivatives (reduction of cytotoxicity >500 fold).

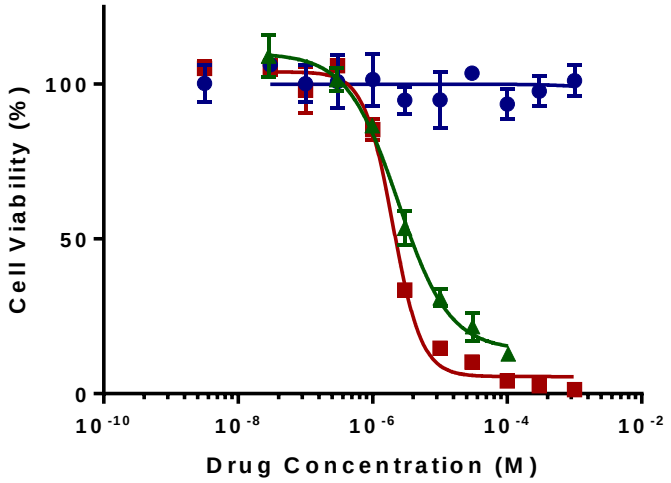
Pro-5FU



COLORECTAL Cancer Cells

	EC_{50}
Pro-5FU	>1 mM
5FU	2.0 uM
Pd + Pro-5FU	2.4 uM

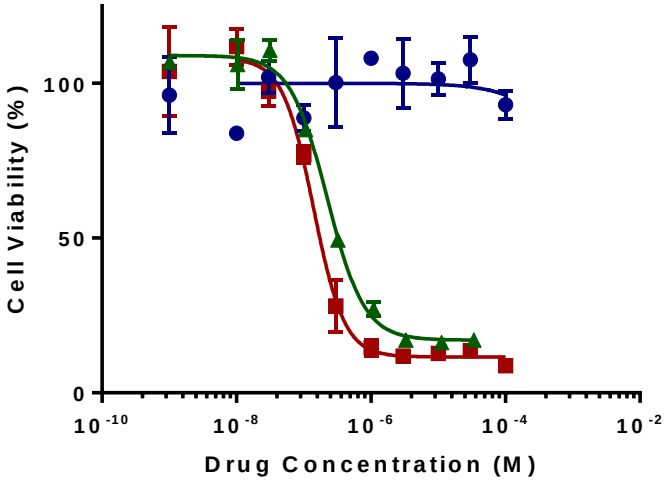
> 500 fold



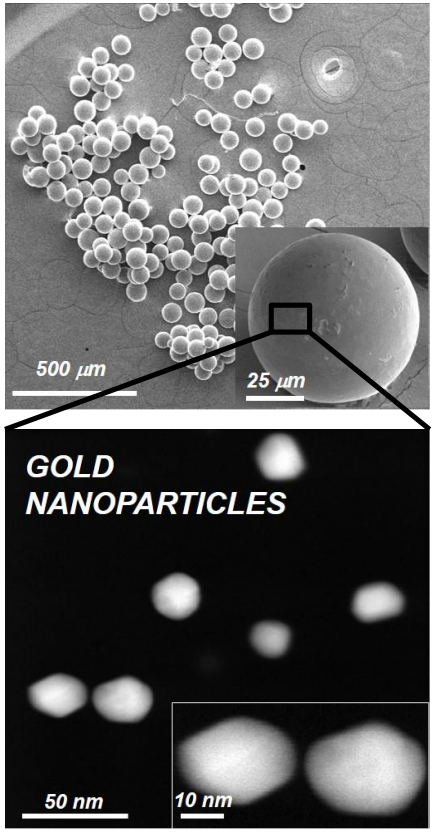
PANCREATIC Cancer Cells

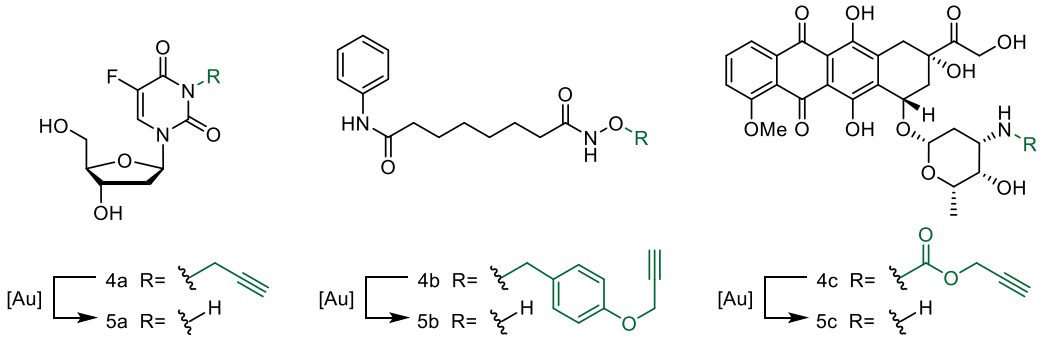
	EC_{50}
Pro-5FU	>100 uM
5FU	136 nM
Pd + Pro-5FU	209 nM

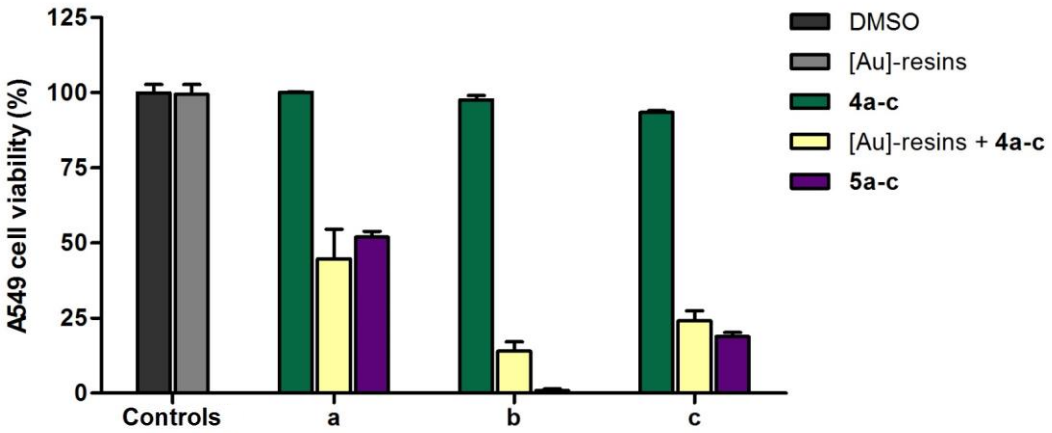
> 700 fold



Beyond Palladium

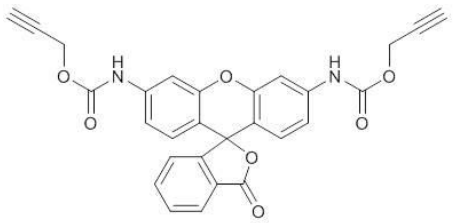






We envisioned that embedding Au-NP in a solid support would serve to protect the metal from large thiol-rich biomolecules, while allowing the free entry of alkyne-functionalized small molecules to undergo gold-mediated chemistries even in biological systems >> ***safe prodrug activation***

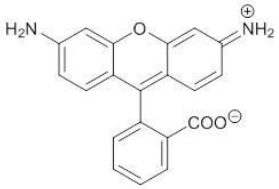
Intracranial Activation of a Pro-dye



Pd-Labile PROBE

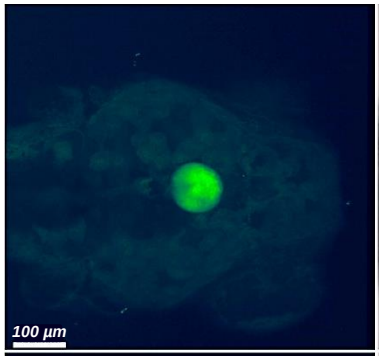
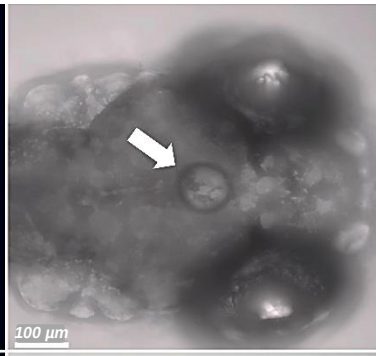
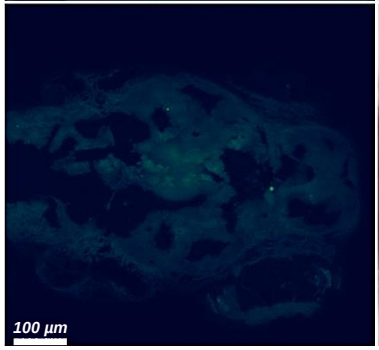
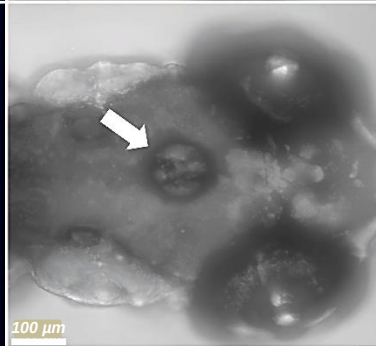
Non Fluorescent / Lipophilic

[Au]-resin  **In the brain of a zebrafish embryo**



Rhodamine 110

Fluorescent / Hydrophilic

	488 nm	***Brightfield***
[Au]-resin + PROBE		
[Au]-resin only		

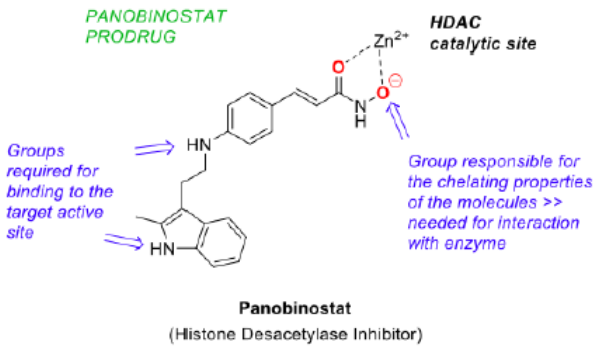
First example of heterogeneous metal-catalyzed release of a chemical reporter performed in the brain of a living animal


THERA
CAT

ESR 5

ESR 5 - EDI	Prodrug design and synthesis	PhD: Yes	Deliv.: 4.1, 4.3	Start date: M6	Duration 36	WP4

Objectives: **1.** Synthesis of prodrugs; **2.** Validation of Pd-mediated drug release in vitro; **3.** Reduction of prodrugs' activity by 100-fold relative to parent drug; **4.** Demonstration of Pd-triggered release of the functional drug in cell culture.



Panobinostat
(Histone Desacetylase Inhibitor)

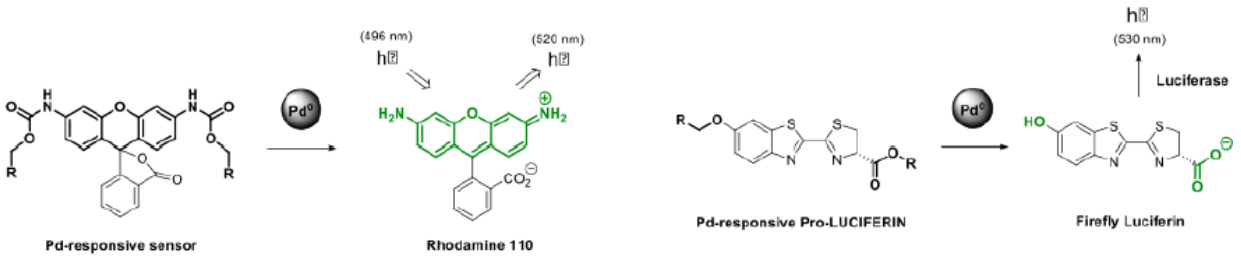
Description: ESR5 will investigate the development of a series of biochemically-stable (= bio-orthogonal) prodrugs specifically designed to become active upon reaction with Palladium (Pd) catalysts. We will generate and test Pd-activated prodrugs using a range of Pd-labile protecting groups. Such studies will enable to expand the arsenal of chemotherapy drugs that can be exploited through this novel spatially-targeted strategy, including therapeutics that are either currently used in the clinic for melanoma and breast cancer treatment. To maximize the clinical impact of the strategy, Pd-labile prodrugs will be developed from a selection of therapeutics with different mode of actions, e.g. HDAC inhibitors (panobinostat), kinase inhibitors (dabrafenib and selumetinib) and alkylating agents (duocarmycin). Prodrugs' sensitivity to Pd will be tested using the methodology developed by ESR6. The efficacy of the deactivation strategy (= bio-orthogonality) will be determined by performing dose response studies with the prodrug and the parent drug in cancer cell lines, which will be followed by the study of the Pd-mediated release of each drug using standard phenotypic assays.

Planned secondments: **TAG** – Pro-imaging PET agents (M24, 4 months); **TAU** – Test micelles catalysts (M34, 3 months).	**Expected results (deliverables)**: Synthesis of 6-10 prodrugs (D4.1); Pd-mediated drug release ranked by reaction kinetics (D4.3); 2-4 prodrugs showing >100-fold reduction in activity (D4.3); Prodrug activation in cell culture (D4.3).

ESR 11 - EDI	Pd in vivo implants and Pd-activatable tools	PhD: Yes	Deliv.: 6.2 - 6.4	Start date: M9	Duration 36	WP6

Objectives: **1.** Synthesis of Pd-implants; **2.** Synthesis of probes; **3.** Validation of Pd-mediated activation in vitro; **4.** In vivo compatibility studies of Pd-implants; **5.** Demonstration of Pd-triggered probe activation in vivo.

Description: The student will develop novel implantable Pd-devices and a range of chemical tools that will allow us to evaluate the catalytic activity of metallic Pd *in vivo* (e.g. surgically-implanted in tumour xenografts or tissues) and expend its scope. To facilitate surgical implantations, Pd-devices of appropriate size (> 4mm) will be developed. ESR11 will investigate the manufacture of larger devices by physically merging them in an appropriate mould. To enable the localised use of naked Pd nanoparticles (NP), a novel technique will be tested in which NP are "bagged" in sealed sachets made out of dialysis tubing. The catalytic capabilities of Pd-implants will be investigated using Pd-responsive sensors prepared from well-established fluorescent, bioluminescent and chemoluminiscent reagents (see Figure) and tetrazines (for click-to-release strategies, ESR12). Masking of reagents' strategic groups will block their reporting properties, which will only be restored upon Pd catalysis (see Figure). In vitro comparative analysis of the probes will allow ranking the best probes for in vivo sensing. In collaboration with consortium partners, animal studies will be performed to determine the compatibility of the devices. After tumour mass formation, devices will be surgically implanted in the tumour and the chosen sensor/protetrazine intravenously-administered. Mice health will be monitored over time and sensor activation analysed by non-invasive in vivo optical imaging.



Planned secondments: **BGX** – gel-based implants (M21, 3 months); **TAU** – in vivo imaging (M36, 3 months).	**Expected results (deliverables)**: Synthesis of Pd-implants (D6.2); Synthesis of 6-8 probes (D6.2); Pd-mediated sensor activation ranked by reaction kinetics (D6.3); 2-4 implants show total biocompatibility (D6.2); In vivo activation of probes / tools (D6.4)

Secondments

Use of our expertise in prodrug design by THERACAT partners

ESR 1 - GRO	**Novel Ru and Pd Complexes of Polypyridine for Catalysis in Living Cells**	**PhD**: Yes	**Deliv.**: 3.1, 5.1	**Start date:** M6	**Duration** 36	**WP3**
ESR 6 - IBEC	**Single molecule imaging of prodyes activation**	**PhD**: Yes	**Deliv.**: 4.2, 4.3	**Start date:** M6	**Duration** 36	**WP4**
ESR 10 - BAS	**Targeting Human Carbonic Anhydrase IX for Drug Release via Metathesis**	**PhD**: Yes	**Deliv.**: 5.1, 5.3	**Start date:** M9	**Duration** 36	**WP5**
ESR 12 - TAG	**in vivo click and click-to-release strategies for catalysts**	**PhD**: Yes	**Deliv.**: 6.3, 6.4	**Start date:** M9	**Duration** 36	**WP6**

1.3. Workplan Tables

1.3.1. WT1 List of work packages

WP Number[9]	WP Title	Lead beneficiary[10]	Start month[12]	End month[13]
WP1	Ethics requirements	1 - IBEC	1	48
WP2	Management and coordination	1 - IBEC	1	48
WP3	Catalysts synthesis	2 - TU/e	6	42
WP4	Prodrugs design and synthesis	5 - EDI	6	42
WP5	In vitro delivery and imaging	1 - IBEC	9	45
WP6	In vivo evaluation	6 - TAU	9	45
WP7	Training	6 - TAU	1	48
WP8	Dissemination and outreach	5 - EDI	1	48

WP4

Work package number [9]	WP4	Lead beneficiary [10]	5 - EDI
Work package title	Prodrugs design and synthesis		
Start month	6	End month	42

Objectives

1. Synthesis of prodrugs; 2. Synthesis of prodyes; 3. Understanding prodrug/dyes activation kinetics, stability and turnover rates.

Description of work and role of partners

WP4 - Prodrugs design and synthesis [Months: 6-42]
EDI
Task 4.1. Synthesis of a library of anti-cancer drugs (e.g. selumetinib and panobinostat) protected with propargyl/allyl groups (EDI).
Task 4.2. Synthesis of fluorescent dyes such (rhodamines, cyanines) protected with propargyl/allyl groups (IBEC).
Task 4.3. Spectroscopic (bulk) and microscopic evaluation (single molecule) study of catalysis (EDI, IBEC).

Participation per Partner

Partner number and short name [10]
1 - IBEC
5 - EDI

WP8

Work package number [9]	5 - EDI
Work package title	
Start month	48

To promote the efficient an… as general public about THERACAT, its training pc…

WP8 - Dissemination and … EDI

Task 8.1. THERACAT web…

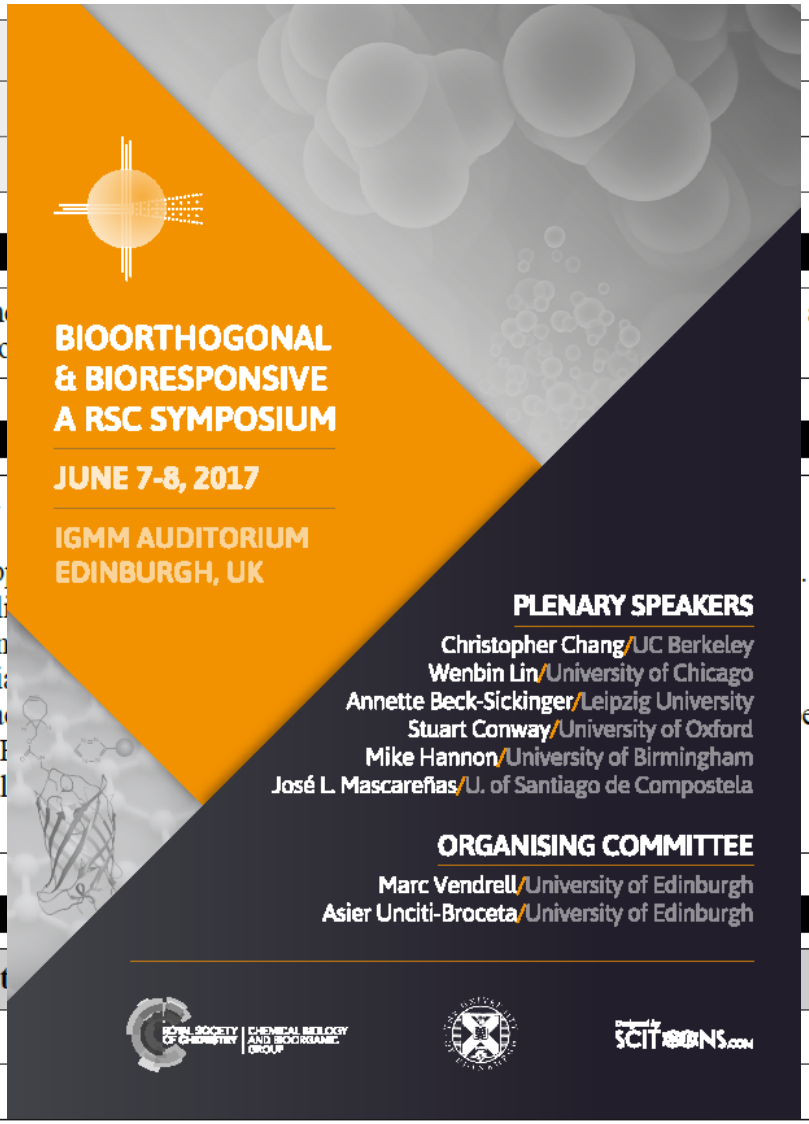
Task 8.2. Open-access publi…

Task 8.3. Work presented in…

Task 8.4. THERACAT socia…

Task 8.5. Communication a… ean Researchers' Night, general press articles, THE…

Task 8.6. ETN international…



Partner number and short…
1 - IBEC
5 - EDI