INNOVATING IN CANCER THERAPY

TALK FOR THERACATS

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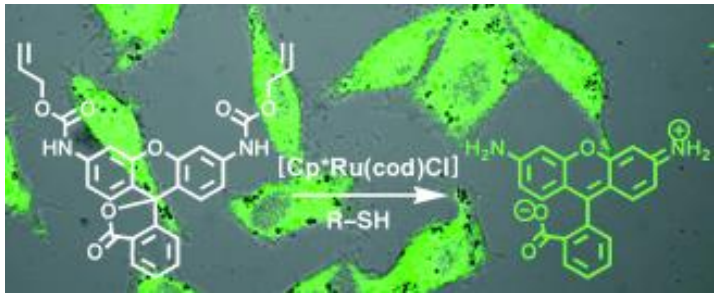
Non-natural metal catalysis in cells



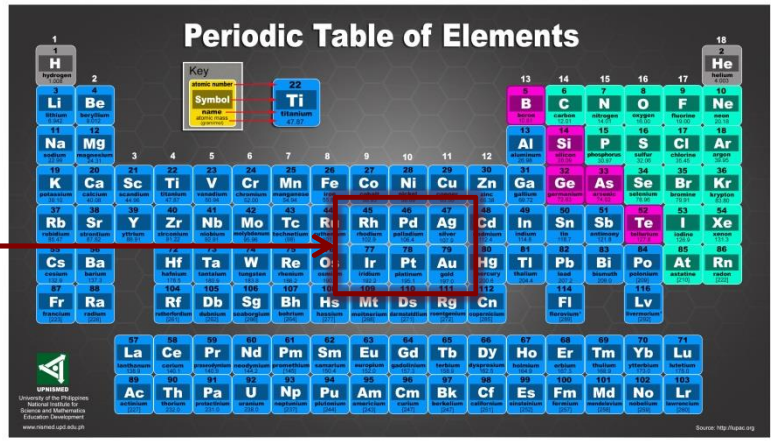
Antecedents: *Meggers developed a water-soluble ruthenium complex that rapidly entered cells and performed an allylcarbamate cleavage, while proving to be non-toxic to cells during the short duration of the experiment (minutes).*

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

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

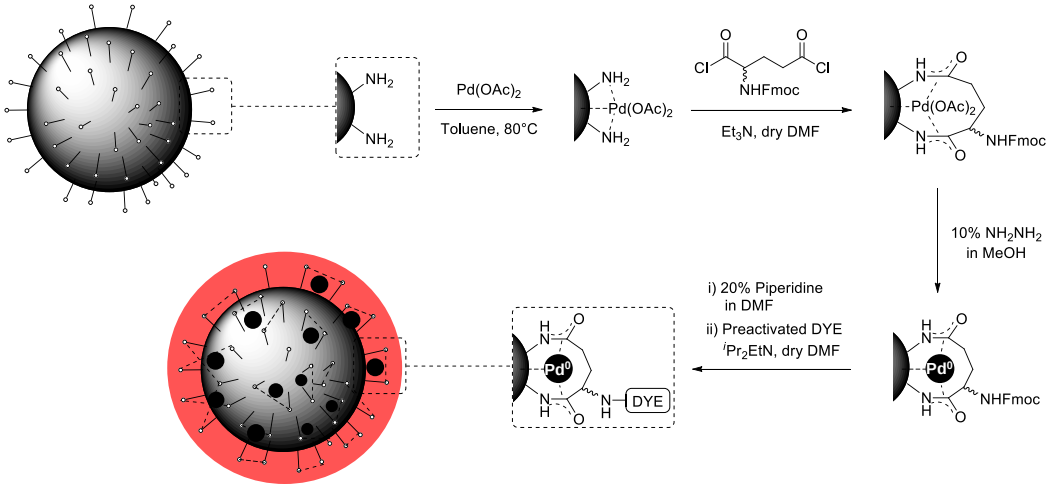
Toxicity & Mechanism:
Metals from the platinum group trigger cell death by cross-linking of DNA >>
HIGHLY TOXIC

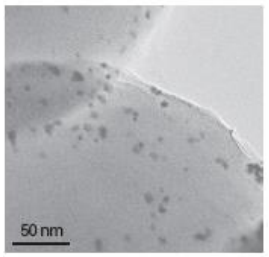
Hypothesis:
Restriction of the catalyst ‘s freedom to enter cell nuclei will suppress its toxicity mechanism >>
HETEROGENEOUS CATALYST

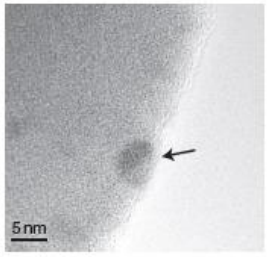


Palladium chemistry in cells

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

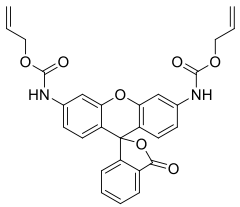






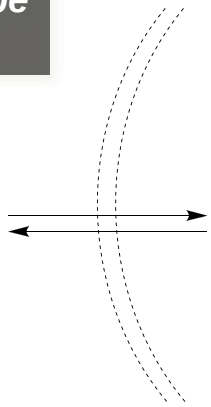
Biocompatible microspheres that penetrate cells and stay in the cytoplasm >> exonuclear location >> minimal toxicity

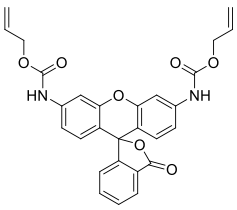
Pd^0 Sensitive Probe (lipophilic)



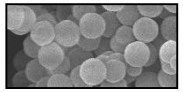
1

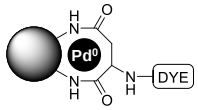
Non-Fluorescent



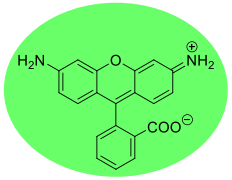


Cytoplasm





Active Dye (hydrophilic)



2

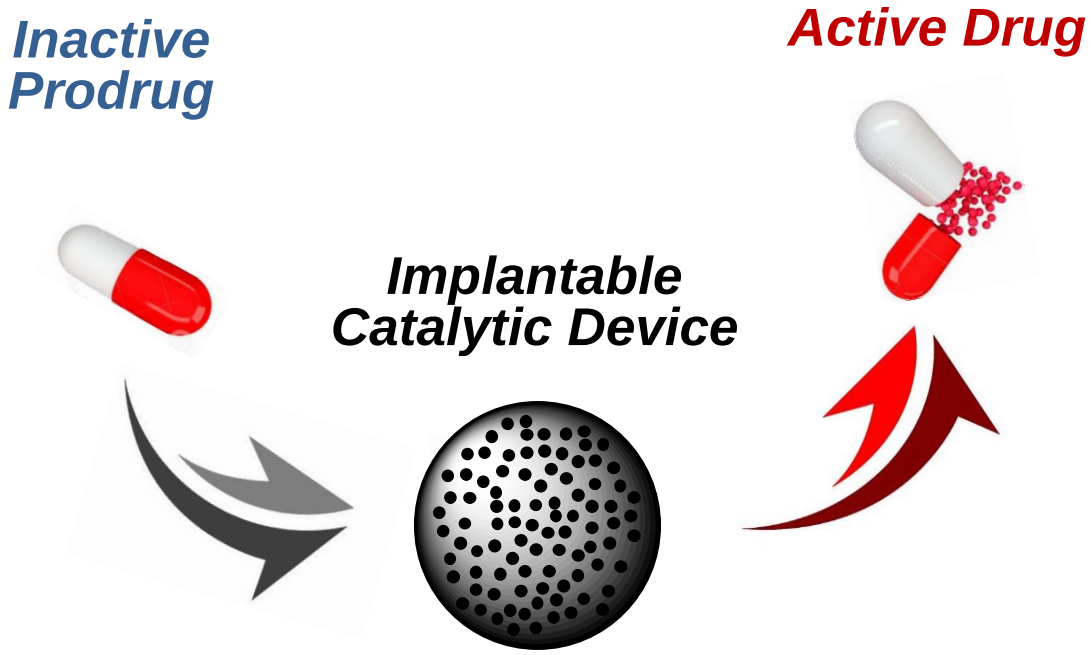
Ex / Em = 498 / 521 nm

Alloc deprotection & Suzuki coupling inside cells

SAFE ON THEIR OWN,

CYTOTOXIC TOGETHER

Implants that make drugs inside your body



GLOCAL Therapy is completely benign until the Prodrug meets the Catalytic Device

→ *Global (systemic) dosing of orally-available Inactive Prodrug >>> ORAL ADMINISTRATION*

→ *Prodrug stable to pH, redox potential and enzymatic metabolism >>> NO SIDE EFFECTS*

→ *Activation only where the catalyst is: Prodrug into Drug >>> LOCAL TREATMENT*

Clinical benefits

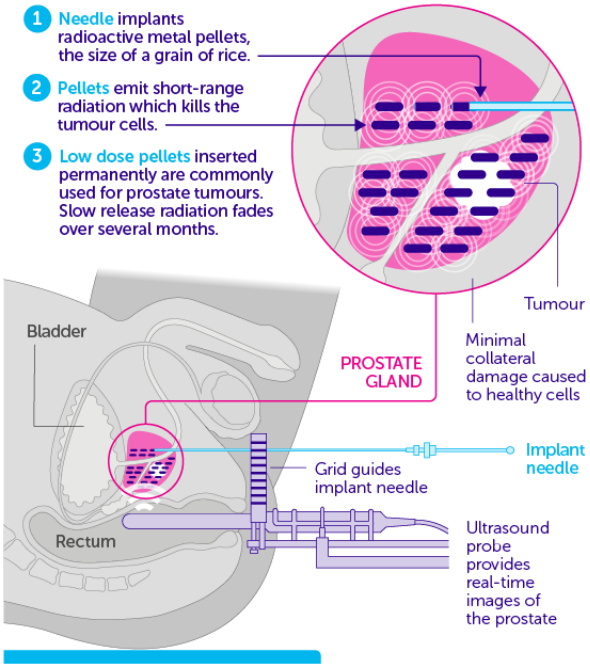
KINDER TREATMENTS

MULTIPLE DRUGS

REPEAT DOSES

***As a catalyst, Palladium can do the job of activating one or more drugs as many times as required**, overcoming the useful life and versatility limitations of other local therapy modalities*

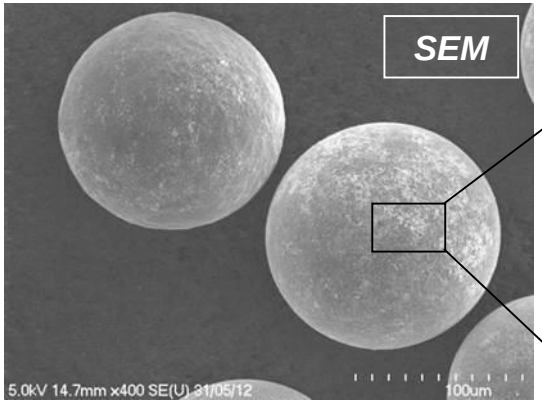
vs BRACHYTHERAPY



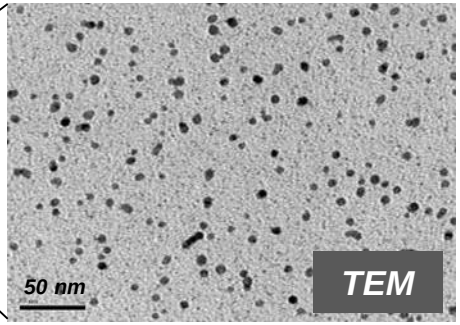
***Importantly, Pd-devices are not radioactive!** Straightforward low-risk implantation procedure for clinicians*

Development of safe Pd-devices

TENTAGEL Resin: *solid support used in conventional solid-phase organic synthesis*

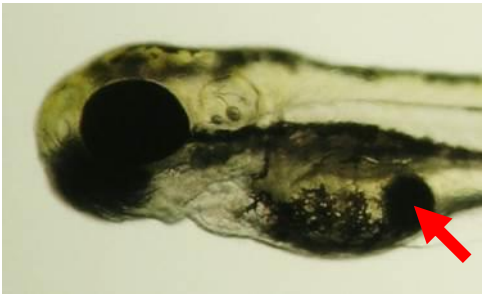


Cross-section

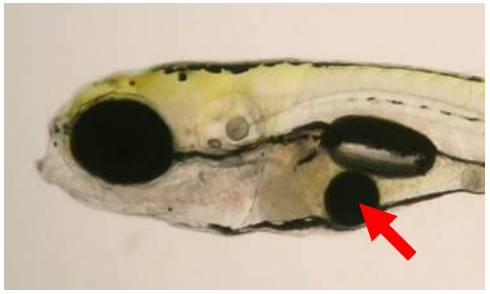


- *Zebrafish embryos (yolk or top of the head) are pierced and a Pd-resin carefully introduced (1 bead / embryo)*
- *Embryo development is monitored for 4 days*
- *Zebrafish embryos containing a **Pd-resin** (indicated with a red arrow) develop normally into their larval stage with no signs of toxicity or alteration of their morphology and behaviour*

24 hpf

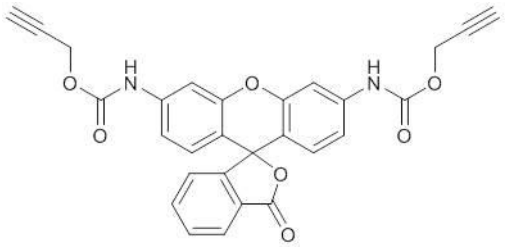


3 dpf



4 dpf

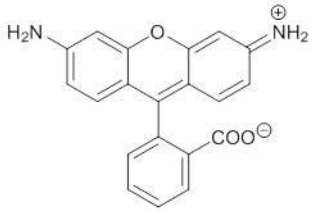
In vivo LOCAL Activation of PROBE



Pd-Labile PROBE

Non Fluorescent / Lipophilic

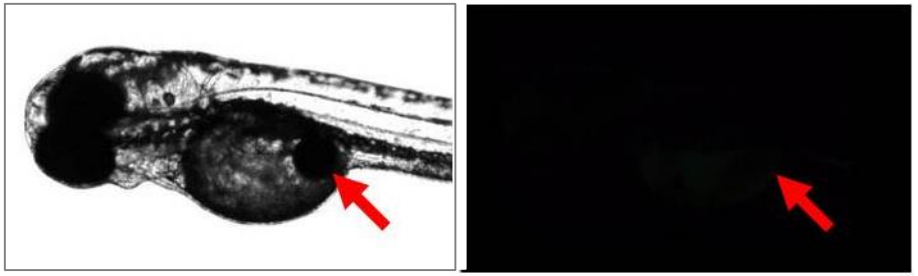
Pd^0-resin (inside yolk of zebrafish embryo)



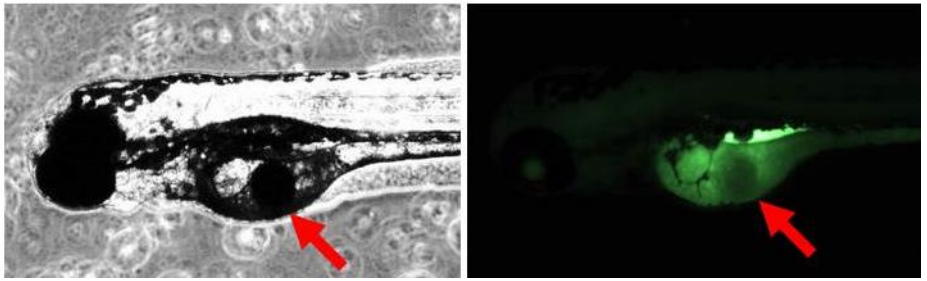
Rhodamine 110

Fluorescent / Hydrophilic

Pd-resin



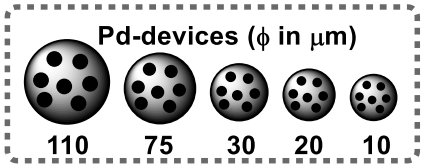
Pd^0-resin + PROBE

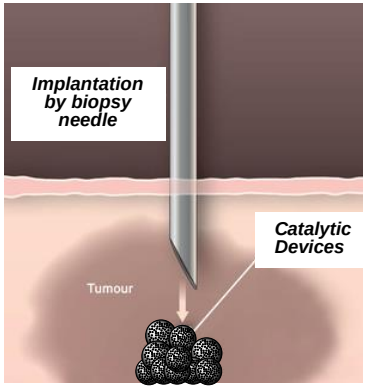


Strong fluorescent signal was clearly observed from the area surrounding the Pd^0-resin in the yolk sac, confirming that the palladium-functionalized device is catalytically active in vivo

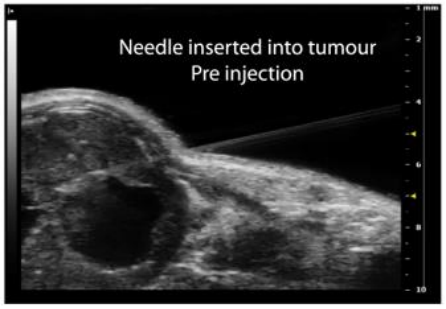
Platform Technology

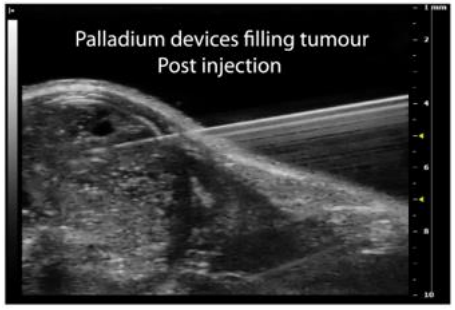
Catalyst		
Palladium	Safe* **Classified as Biocompatible metals*	16w in mice 84d in rats
Gold		





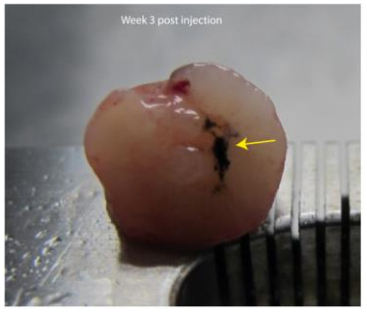
Chemotherapy	Main Cancer Indication
5FU	Colorectal, Breast, Pancreatic, Oesophageal, Vaginal, Cervical, Anal
Irinotecan	Colorectal, Lung, Pancreatic, Head & Neck
Doxorubicin	Breast, Pancreatic, GI, Bladder, kidney, Bone, CNS, Colorectal





Devices are *echogenic* >> intratumoural insertion guided by ultrasound imaging

Devices do not “move” from the point of injection



How to make a bioorthogonal prodrug

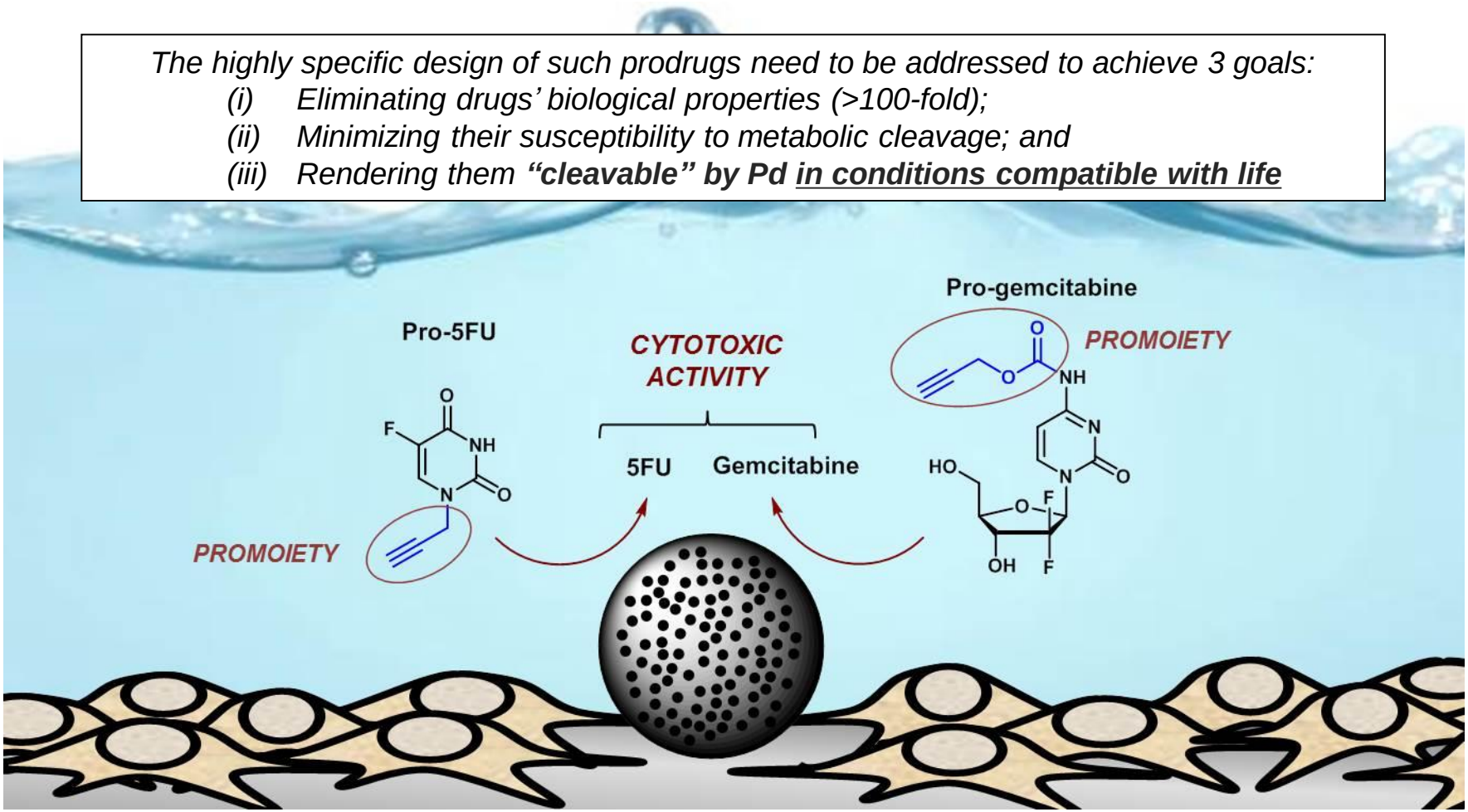
OBJECTIVE: *Control the activation of prodrugs* ***exclusively*** *by implant-localized palladium catalysis*

The highly specific design of such prodrugs need to be addressed to achieve 3 goals:

(i) Eliminating drugs' biological properties (>100-fold);

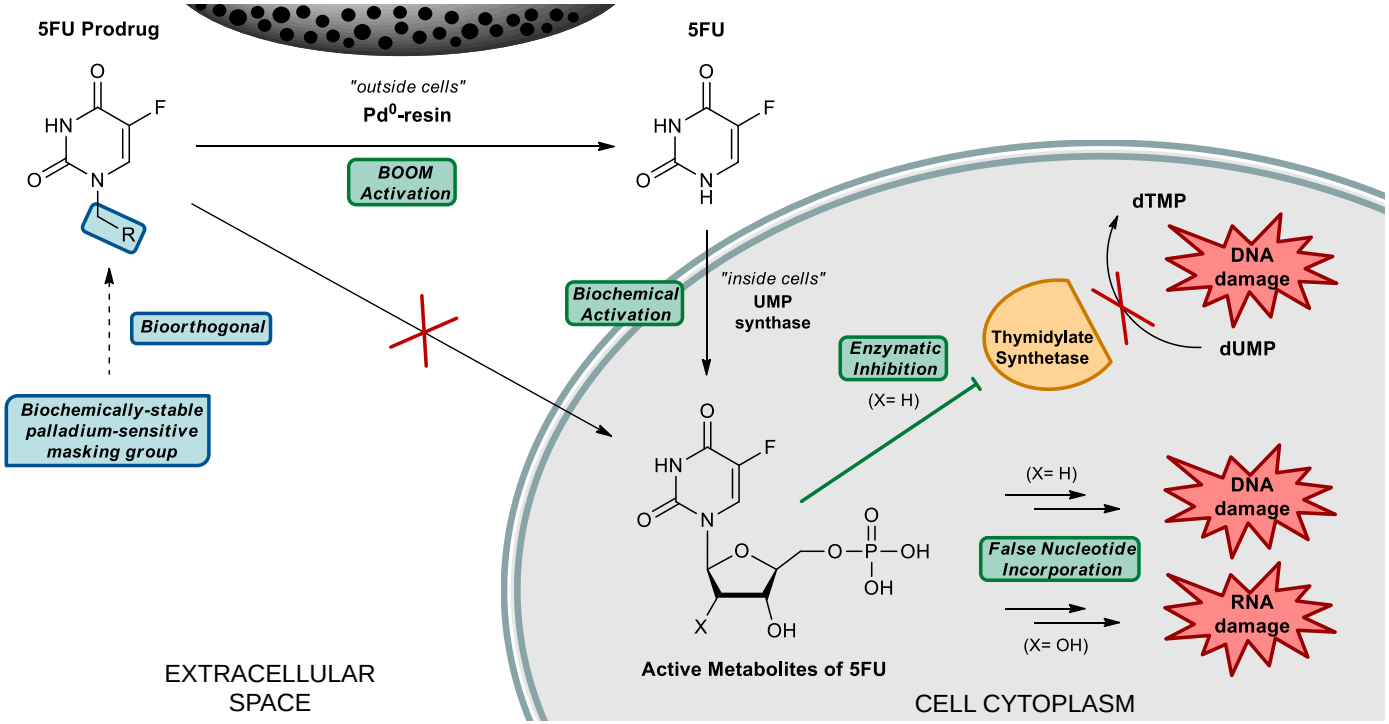
(ii) Minimizing their susceptibility to metabolic cleavage; and

(iii) Rendering them ***"cleavable" by Pd*** ***<u>in conditions compatible with life</u>***



5FU Prodrug Design

GOAL: *To increase prodrug stability using masking groups that are not recognized by hydrolytic enzymes while being labile to Pd chemistry*



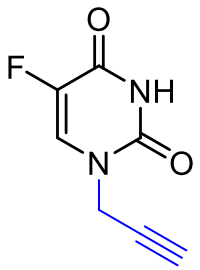
DRUG's Mode of Action:
5FU is converted intracellularly into cytotoxic nucleotidic metabolites, which inhibit directly thymidylate synthase or incorporates into RNA and DNA to disrupt normal cell functions

CHALLENGE: *According to the literature, palladium-mediated N-dealkylations in water typically require temperatures incompatible with cell survival (>80 ° C)* ***KEY:*** *lactam – lactim tautomery*

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).

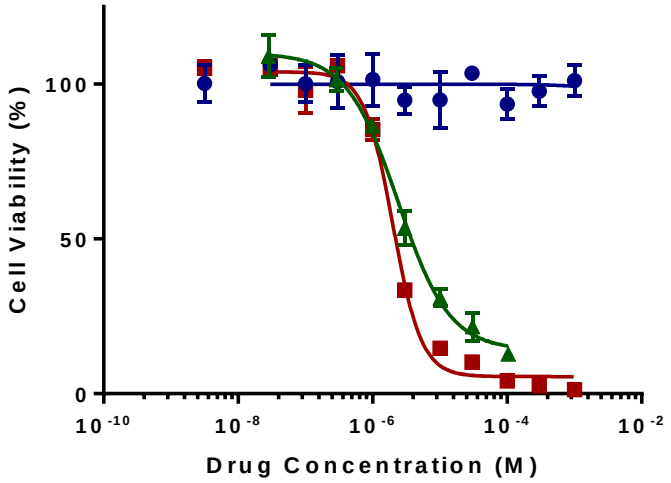
Pro-5FU



COLORECTAL Cancer Cells

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

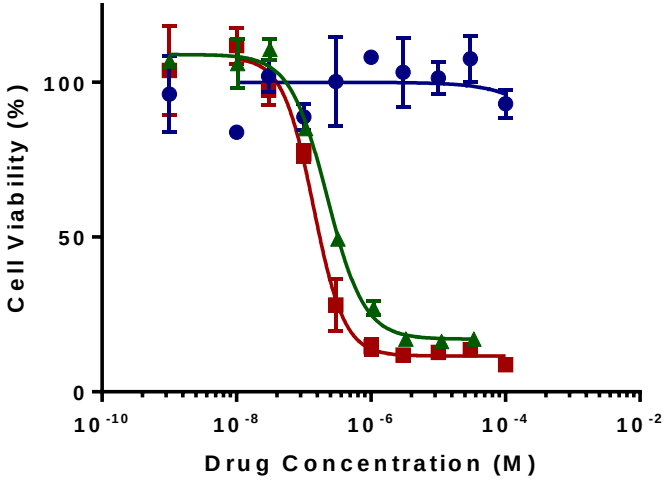
> 500 fold



PANCREATIC Cancer Cells

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

> 700 fold

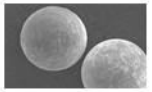


Visualizing inactive prodrug and devices

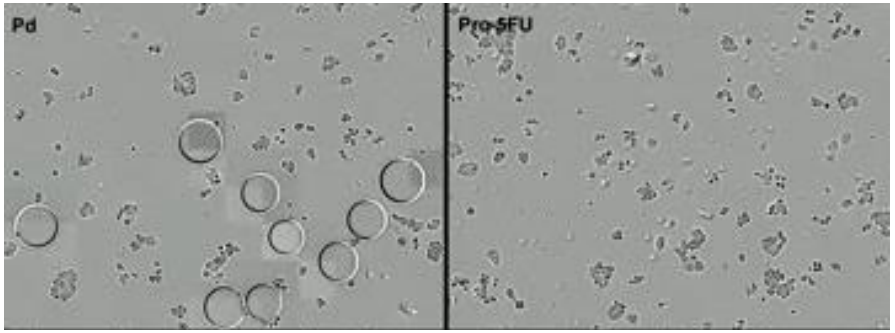
*Chemical masking of **5FU** (drug used to treat colon and pancreatic cancer) results in a completely inactive derivative (reduction of cytotoxicity >500 fold).*

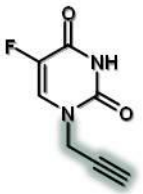
Palladium

SAFE



Pd^0-resins





Pro-5FU

PRODRUG

SAFE

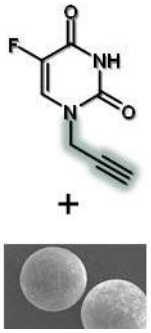
Real-time visualization (5 days) of cell proliferation (colorectal cancer cells)

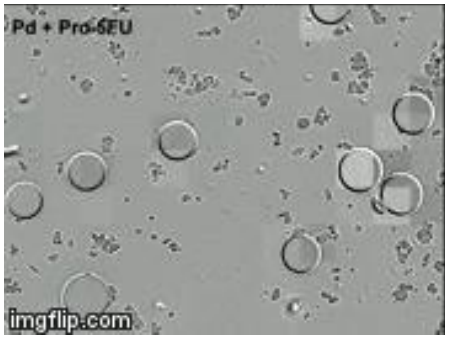
Nat. Commun. **2014**, 5, 3277

Visualizing prodrug activation

*Combination of inactive **Pro-5FU** and **Pd-devices** mediated strong cytotoxic activity, equivalent to that of **5FU**, demonstrating the in situ manufactured of the drug*

PRODRUG + Palladium

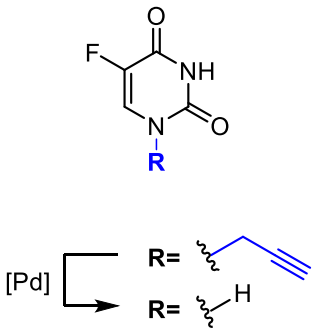




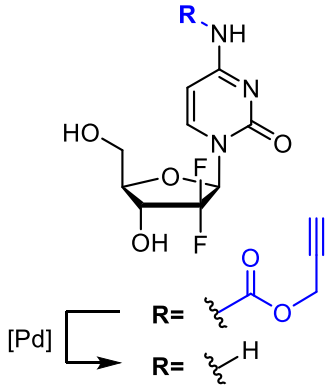
CYTOTOXIC

Real-time visualization (5 days) of cell proliferation (colorectal cancer cells)

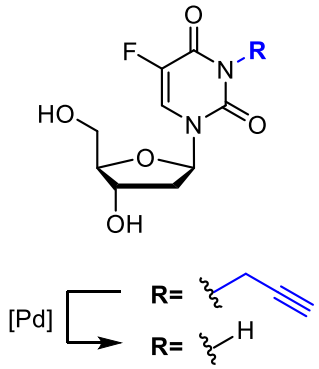
Palladium activated prodrugs



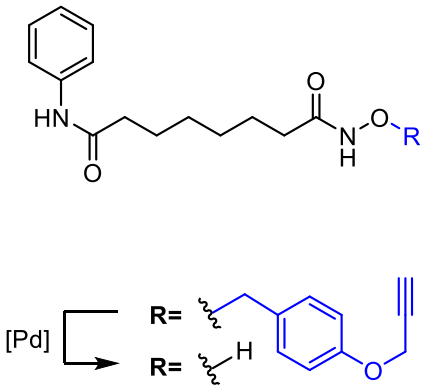
Nat. Commun. **2014**, *5*, 3277



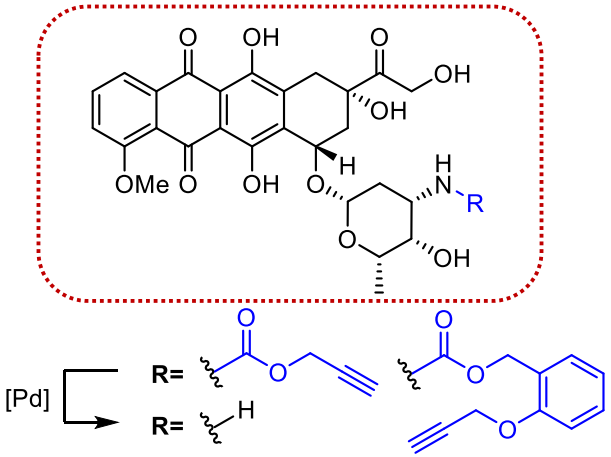
J. Med. Chem. **2014**, *57*, 5395



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

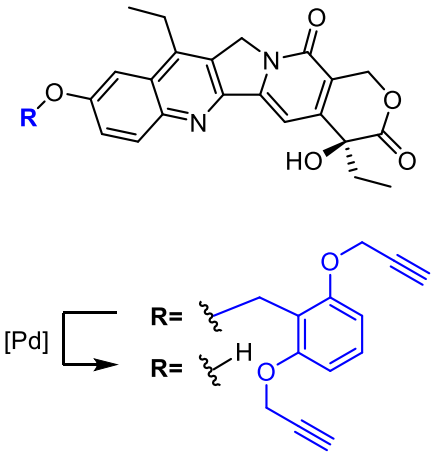


J. Med. Chem. **2016**, *59*, 9974



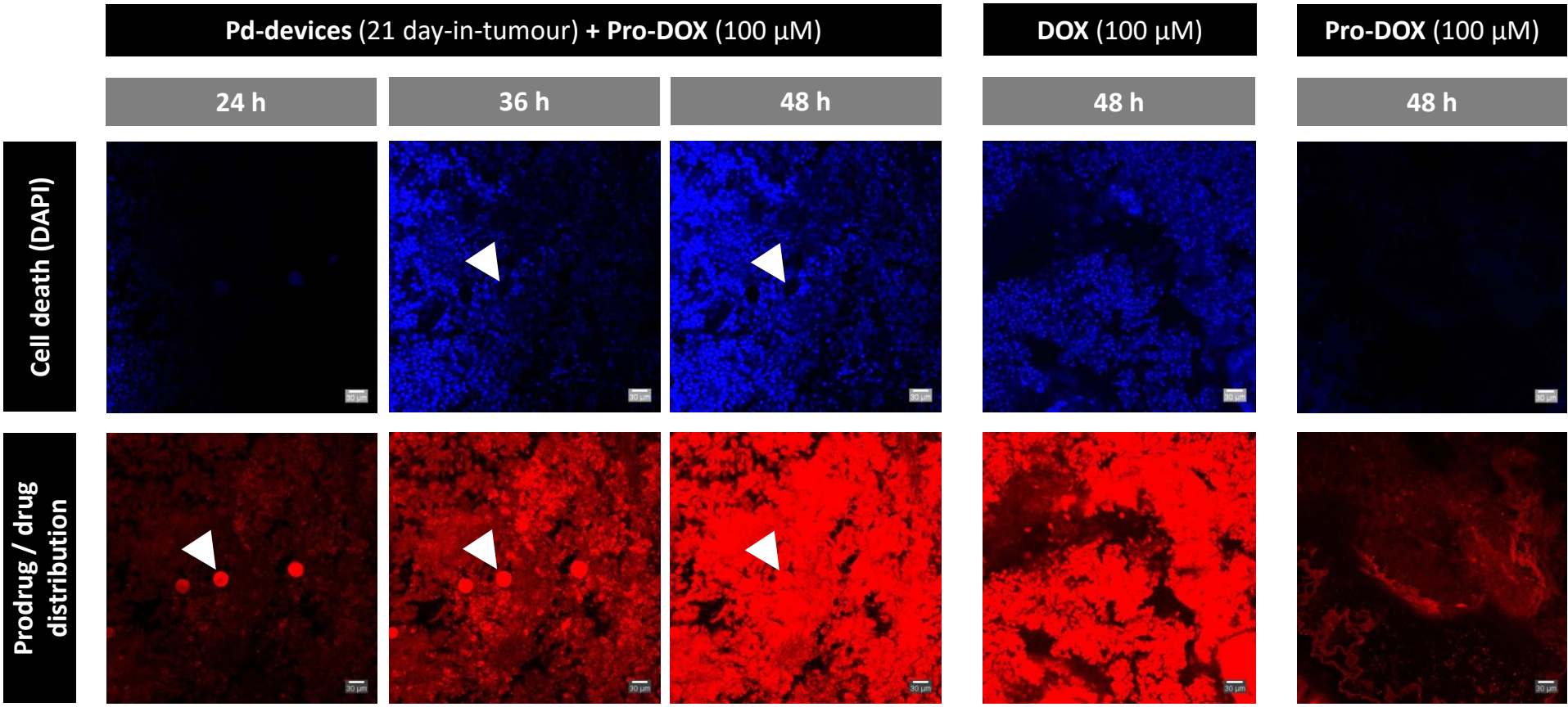
Angew. Chemie **2017**, *56*, 12548

Chem. Sci. **2018**, *9*, 7354-7361



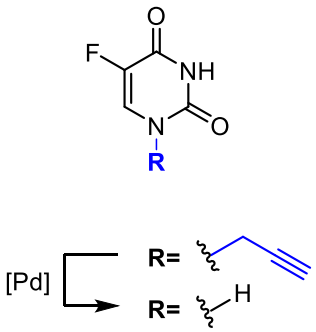
Chem. Eur. J. **2018**, *24*, 16783-16790

Ex vivo activation of chemotherapy

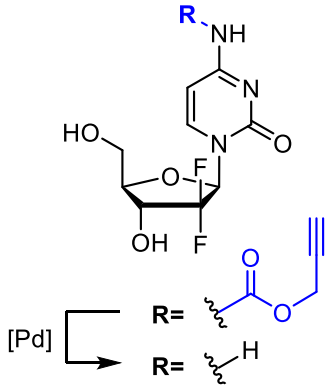


***Ex vivo* Pd-mediated release of DOX from an inactive precursors in a prostate tumour explant**

Palladium activated prodrugs



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



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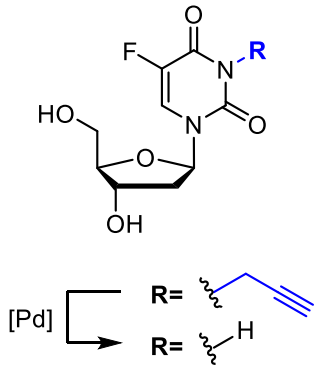

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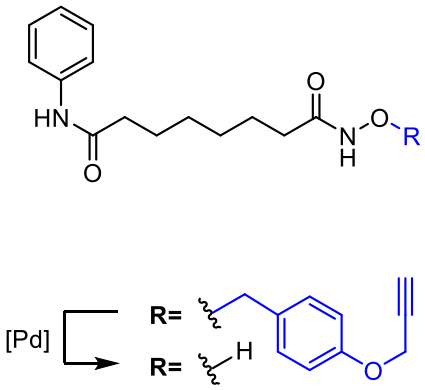
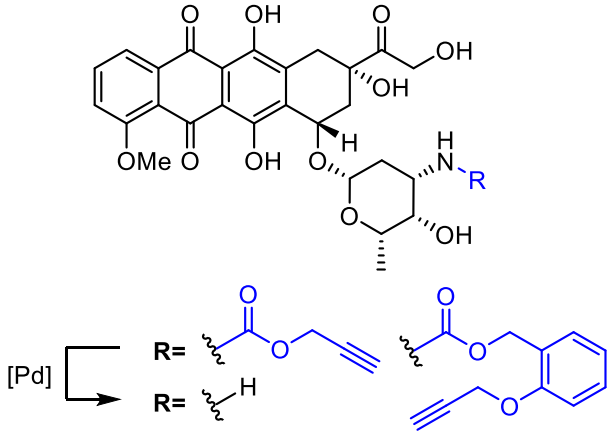


J. Med. Chem. **2016**, *59*, 9974



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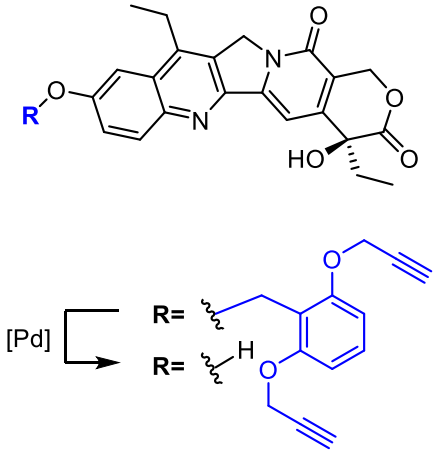

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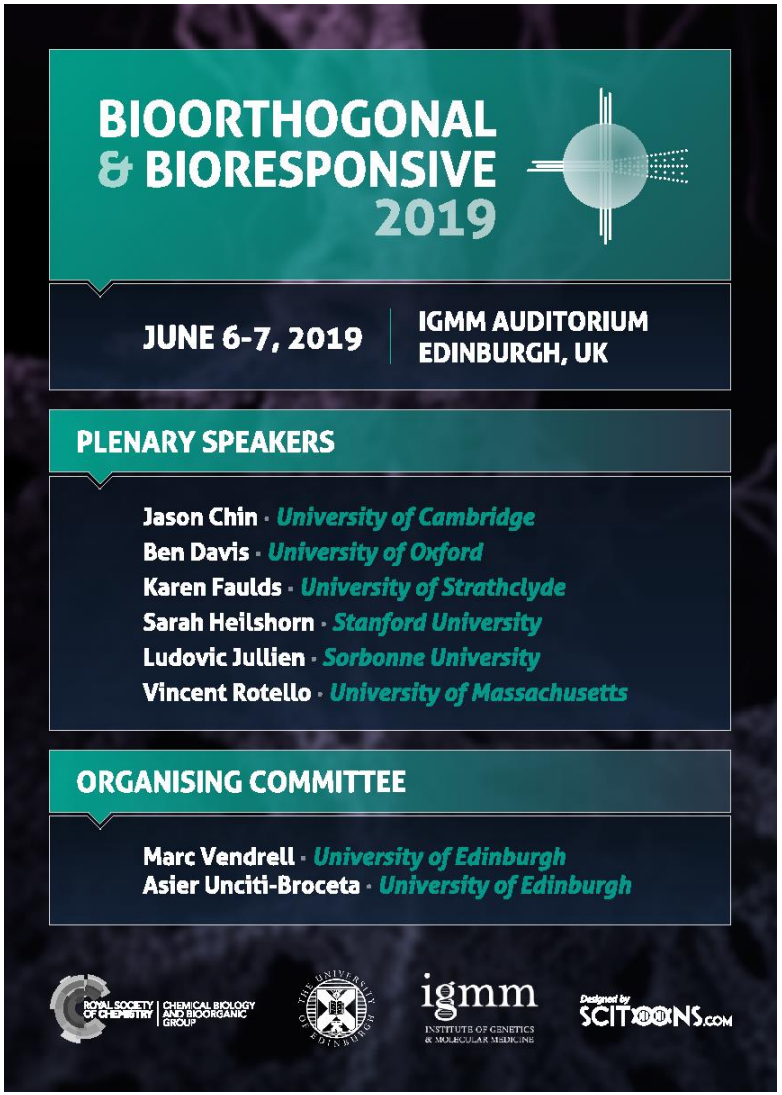

PLEASE DO NOT FORGET TO REGISTER BEFORE FRIDAY TO TAKE ADVANTAGE OF THE EARLY BIRD REGISTRATION!!!

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Zaragoza, Spain, 16-18 October

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FROM THE IGMM SITE

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Val Brunton & Margaret Frame – Mouse cancer models & cell biology

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