Using Photons to Image and Destroy Cancer - Exploiting the Interface of Organic Synthesis, Engineering and Biology

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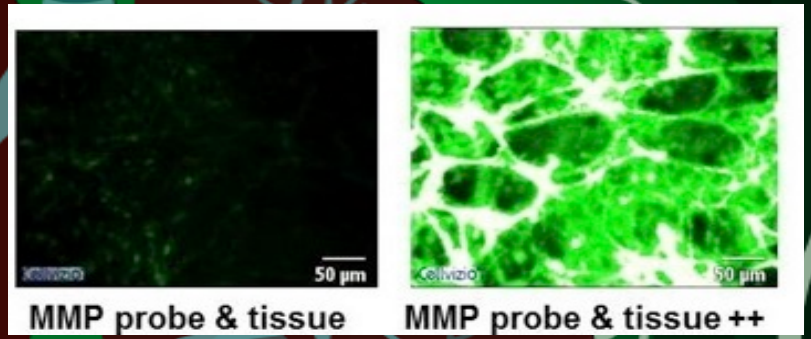
Contents – Two/Three Vignettes

(i). The design and application of chemical probes that can paint disease (cancer);

(ii). Novel CMOS SPAD detectors (targeting cancer margin detection);

(iii). Gamma/X-ray prodrugs

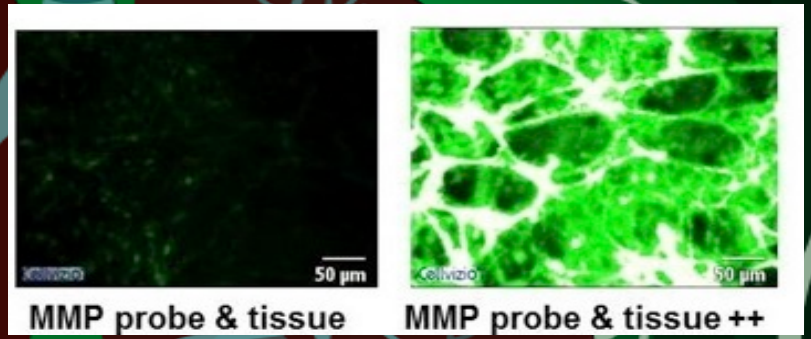
Our Approach - Optical Molecular Biopsy in vivo in situ



- Optical fibre inserted into lung of patient - measure pH or
- Chemical Probe sprayed at low dose (< 100 µg)
- Imaging system (Cellvizio) to allow response at a molecular/cellular level to be measured

Thanks to Helen Parker for the image

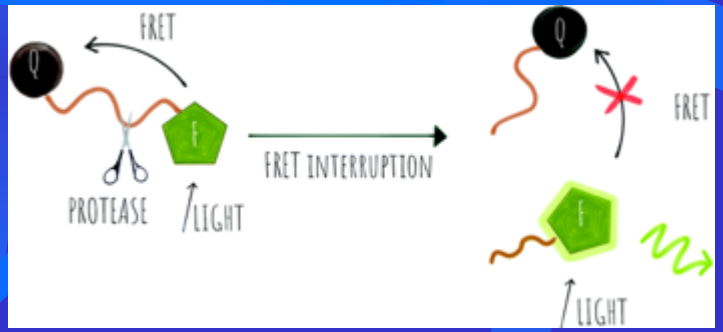
Our Approach - Optical Molecular Biopsy in vivo in situ



- Probes need to have very low background signals
- Activate quickly
- Generate high signals

Fluorogenic Peptide Probes for Proteases

- Proteases are enzymes with wide-ranging and crucial activities that mediate the hydrolysis of amide bonds.
- Proteases are excellent disease biomarkers (many drugs are based on protease inhibitors
- Fluorogenic peptide-based probes allow evaluation of proteolytic activity.



- These probes contain a peptide-moiety that drives specify (i.e. a protease substrate) and a reporter (i.e. a fluorophore)

Lock-Down" project

41 pages
27 figures

Chem Soc Rev

ROYAL SOCIETY OF CHEMISTRY

REVIEW ARTICLE

Peptide probes for proteases – innovations and applications for monitoring proteolytic activity

Maria Rodriguez-Rios, Alicia Megia-Fernandez, Daniel J. Norman and Mark Bradley *

Check for updates

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rsc.li/chem-soc-rev

Proteases are excellent biomarkers for a variety of diseases, offer multiple opportunities for diagnostic applications and are valuable targets for therapy. From a chemistry-based perspective this review discusses and critiques the most recent advances in the field of substrate-based probes for the detection and analysis of proteolytic activity both *in vitro* and *in vivo*.

Introduction

Proteases are enzymes with wide-ranging and crucial activities that mediate the hydrolysis of amide bonds. The residues within the active site of the enzyme responsible for the biological activity allow the partitioning of these enzymes into the five major classes: so-called serine, threonine, cysteine, aspartic acid or metallo-based proteases. Cleavage by proteases typically occurs on well-defined substrates to allow for discrete and precise regulation of biological functions and takes place between two amino acids (P1–P1′) (by endopeptidases) or at the N- or C-terminus of the peptide (by exopeptidases). Proteases need to recognise their substrate to allow exertion of its function, with the most crucial amino acid being that located on the P1 position of the scissile bond. The degree of substrate specificity is variable, with some proteases accepting a broad spectrum of substrates and others accepting very few.

The function of proteases is essential for an abundance of cellular processes, that includes matrix remodelling by metalloproteinases during cell growth, angiogenesis and tissue remodelling;[1] regulation of cell division by the calpain family[2] and the control of programmed cell death by caspases.[3] These physiological proteolytic functions are tightly

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Maria R. Rios

Maria Rodríguez-Ríos received her bachelor's degree in Biochemistry from the Universitat Autonoma de Barcelona in 2015. She obtained a MSc in Drug Design and Biomedical Sciences from Edinburgh Napier University, working on the development of optical probes for cancer imaging. Following a short period working in industry, she started her PhD studies at the University of Edinburgh under the supervision of Professor Mark Bradley working on the synthesis of fluorogenic peptide-based probes for imaging inflammation.

Alicia Megia-Fernandez

Alicia Megia-Fernandez, MRSC, obtained her MSc degree in Chemistry and PhD from the University of Granada, Spain, under the guidance of Professor Santoyo-Gonzalez, where she was awarded the Extraordinary PhD Award in Experimental Science. This was followed by the award of Fundación Ramón Areces and Marie Curie IEF Fellowships to work as a postdoctoral researcher at the University of Edinburgh with Professor Bradley. In 2022 she was appointed to a Maria Zambrano Senior Fellowship at the University of Granada. Her current research interests are in Chemical Biology and include chemical methodology and molecular imaging probes for diagnosis and treatment of different diseases.

MMP Probes (9 and 13)

(IPF and Cancer)

WO 2016/1511297 and BME Frontiers 2021 (Alicia Megia-Fernandez and Sunay Chankeshwara)

Gen 1.0 - MMP-9 Probes – Linear FRET's

FAM-PEG$_2$-(AA)$_n$-K(MR)-NH$_2$
-P-Cha-**G**-**M**-F-G-
-P-F-**G**-**M**-K-ßA-
-P-Cha-**G**-**M**-W-G-
-P-Cha-**G**-**M**-Y(Me)-G-
-P-Cha-**G**-**M**-Y-G-
-P-Cha-**G**-**M**-K-ßA-G-
-P-Cha-**G**-**M**-H-G-
-P-Cha-**G**-**M**-K-G-
-G-P-K-**G**-**L**-K-G
-G-P-K-**G**-**Ile**-K-G
-G-P-K-**G**-**Nle**-K-G
-P-F-G-L-K-ßA-
-P-F-**G**-**Ile**-K-ßA-
-P-F-G-Nle-K-ßA-

Analysis of

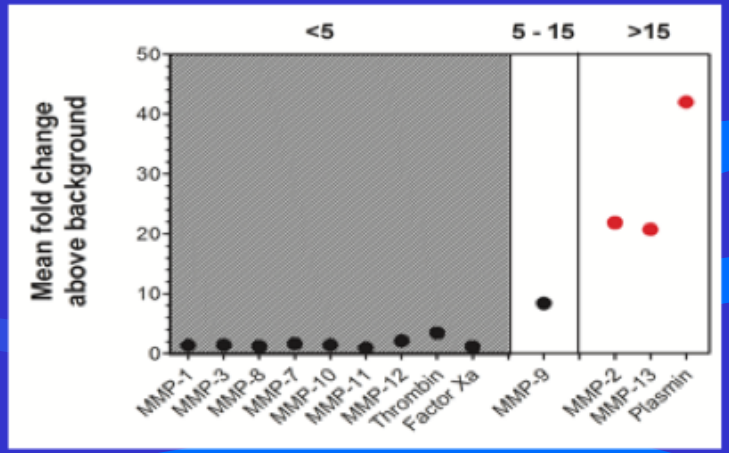
- *Response to MMP-9 and MMP-13*
- *Selectivity vs other MMPs, elastase, macrophages, tissue homogenate, plasmin & Factor Xa*
- *Probe stability in lung lavage*
- *Specific inhibition of signal*
- *Ex-vivo tissue response*

FAM-PEG$_2$-**G-P-K-G-L-K-G**-K(MR)-NH$_2$

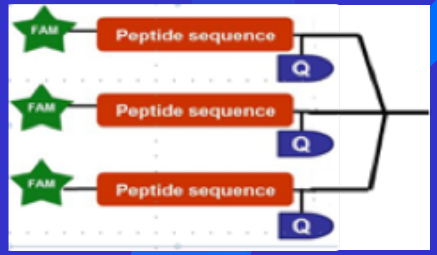
FAM-PEG$_2$-**P-F-G-Nle-K-ßA**-K(MR)-NH$_2$

Specificity of Soluble Lead Probes

AMF-106 - 1µM

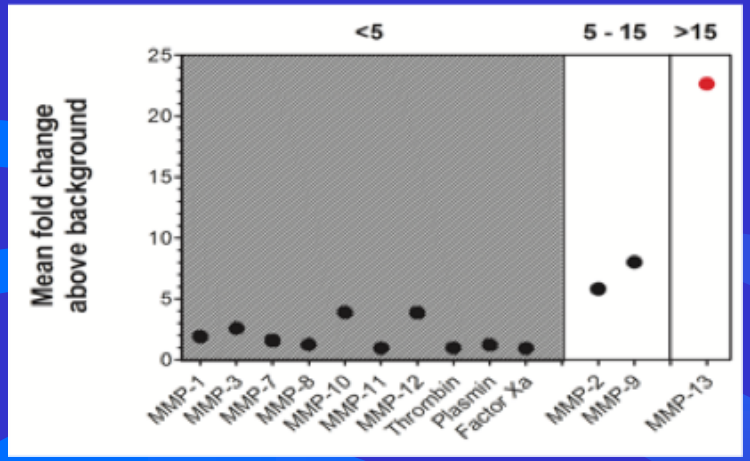


Plasmin > MMP-2 > MMP-13 > MMP-9

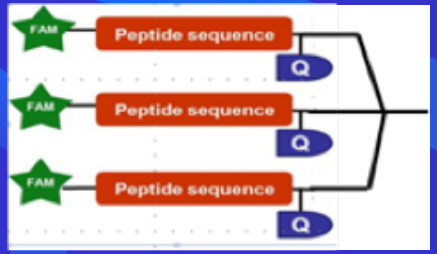


-G-P-K-G-L-K-G-

VC-186 - 1µM



MMP-13 > MMP-9 > MMP-2

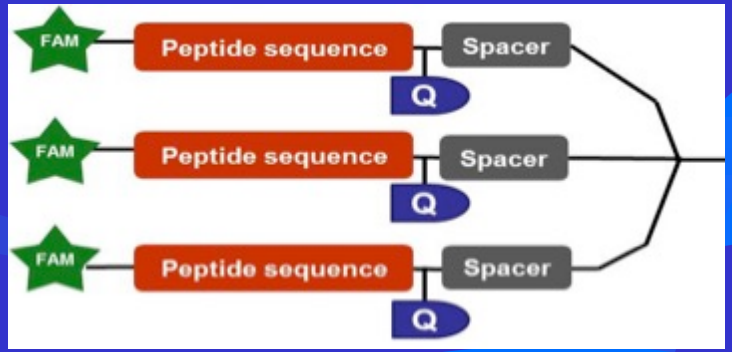


= MMPX

P-F-G-Nle-K-ßA

Optical Imaging Probes for MMP-13 and 9

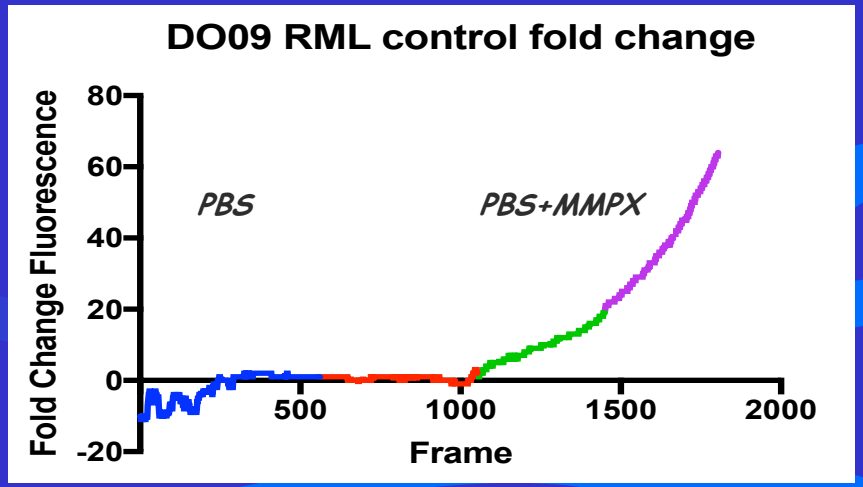
FAM-P-F-G-Nle-K-ßA-K(Quencher)-[PEG_2-k]$_2$

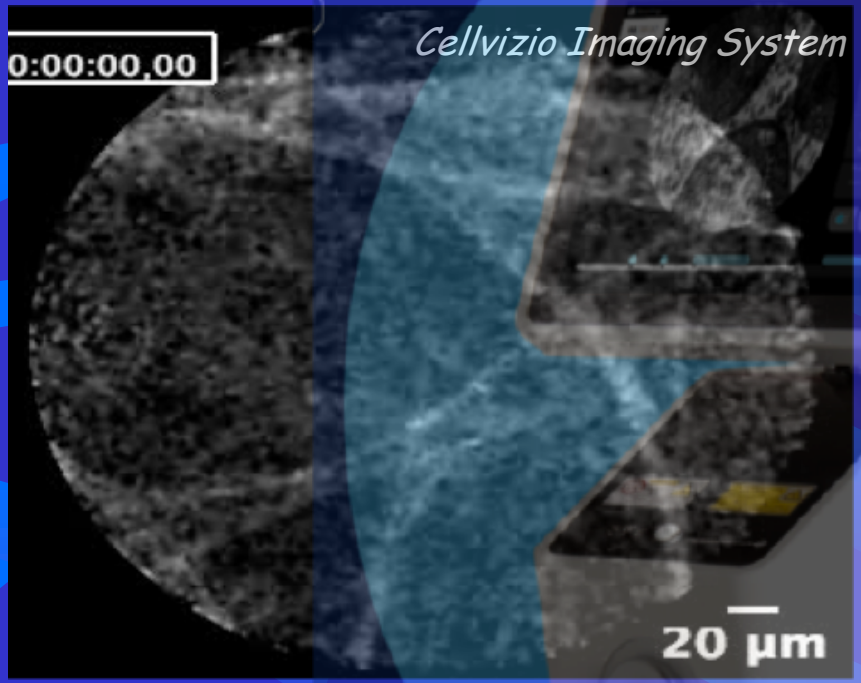


- ✓ 100 MMP2/9/13 probes synthesised and evaluated
- ✓ Tri-branched FRET probe was the best
- ✓ Novel peptide sequence
- ✓ Works in ex-vivo tissue
- ✓ Non-toxic in mice
- ✓ Stability: 1 month @25^{0}C and 6 months@4 °C - Made to GMP
- ✓ Tested in 13 patients with severe lung scarring/cancer

WO 2016/1511297 and
BME Frontiers 2021

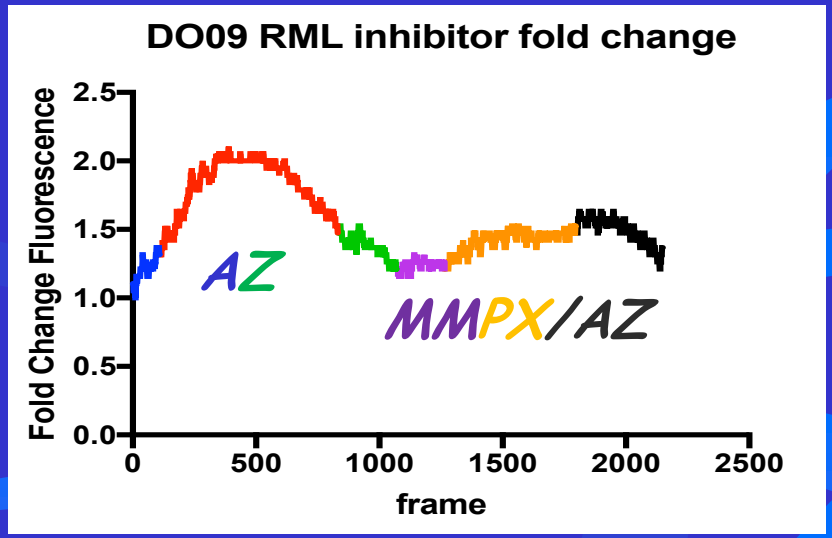
MMP Activity in situ in vivo

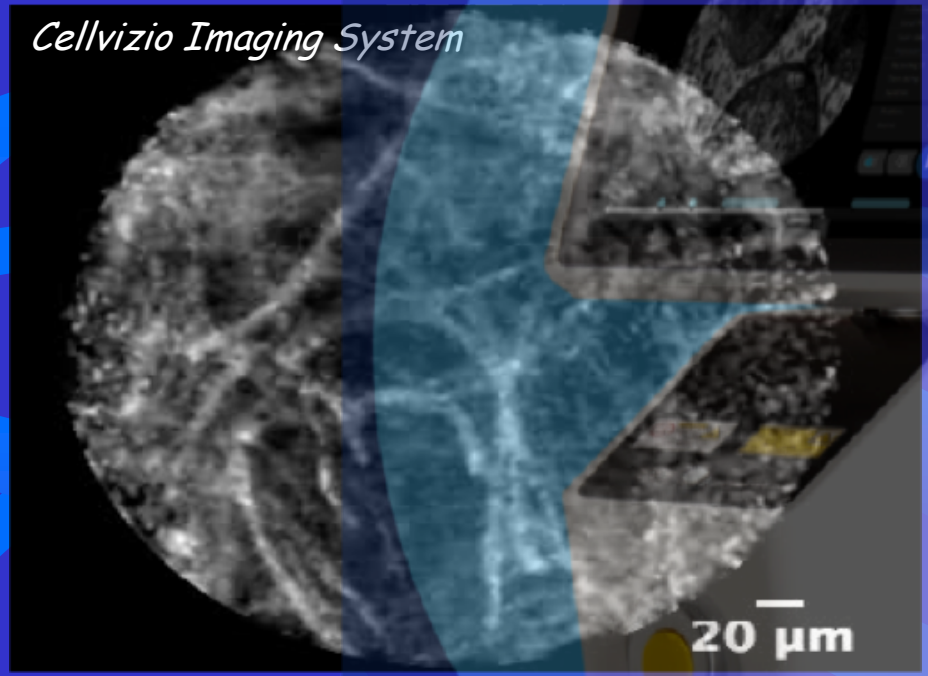




- The alveolar space of a patient after addition of the MMPX probe.
- There is an obvious increase in fluorescence within the lung (which we do not see in healthy lung tissue).

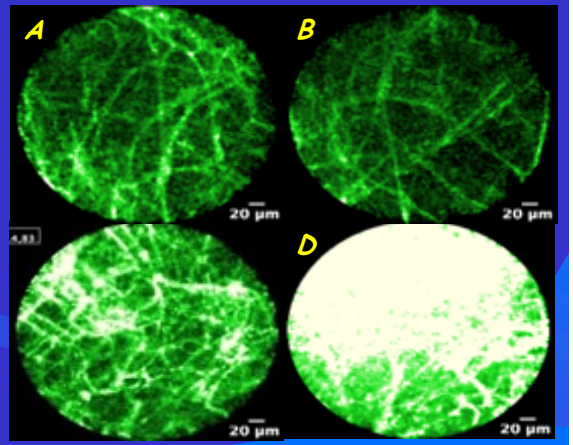
AZ Drug Blocks MMP in situ in vivo

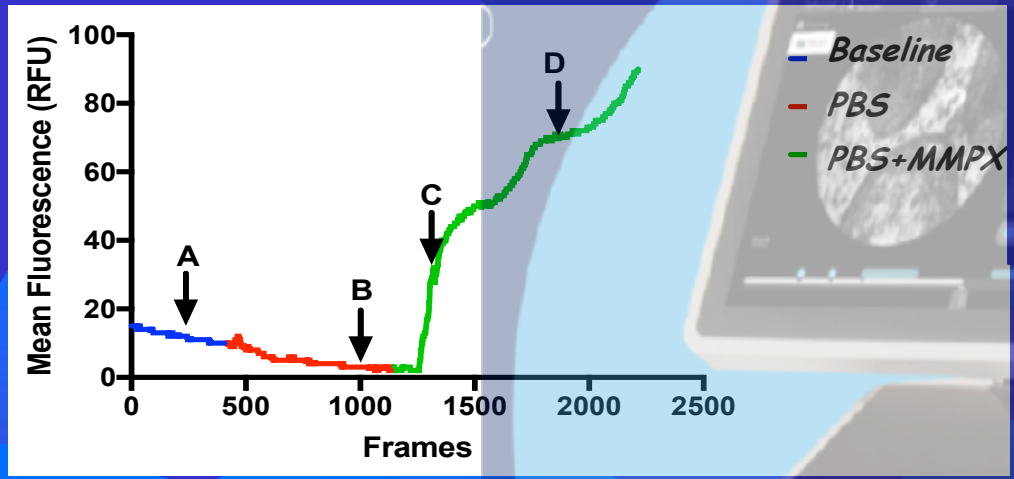


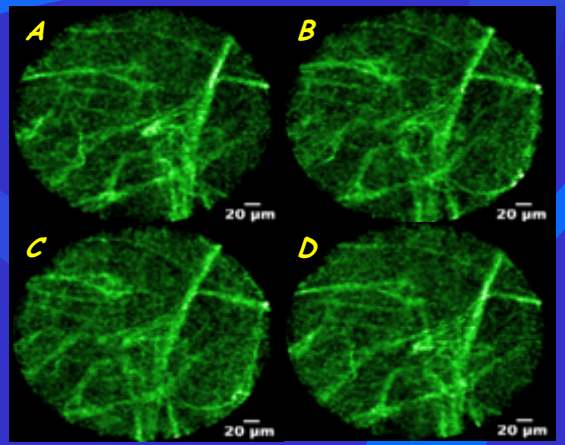


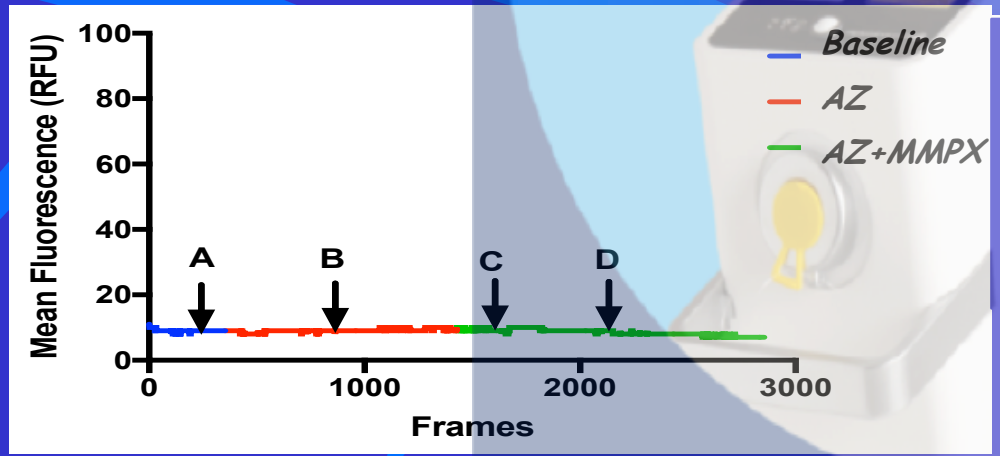
*Reformulated orally bioavailable small molecule inhibitor of MMPs (AZD1236)

Drug-Target Engagement in Tissue - AZ Drug

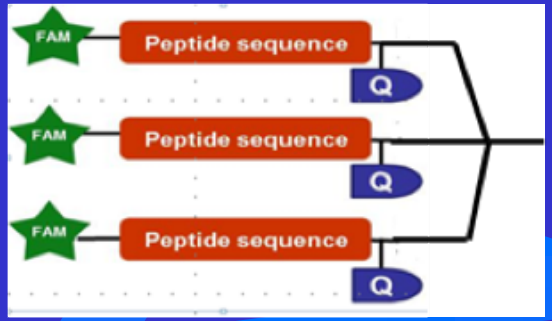






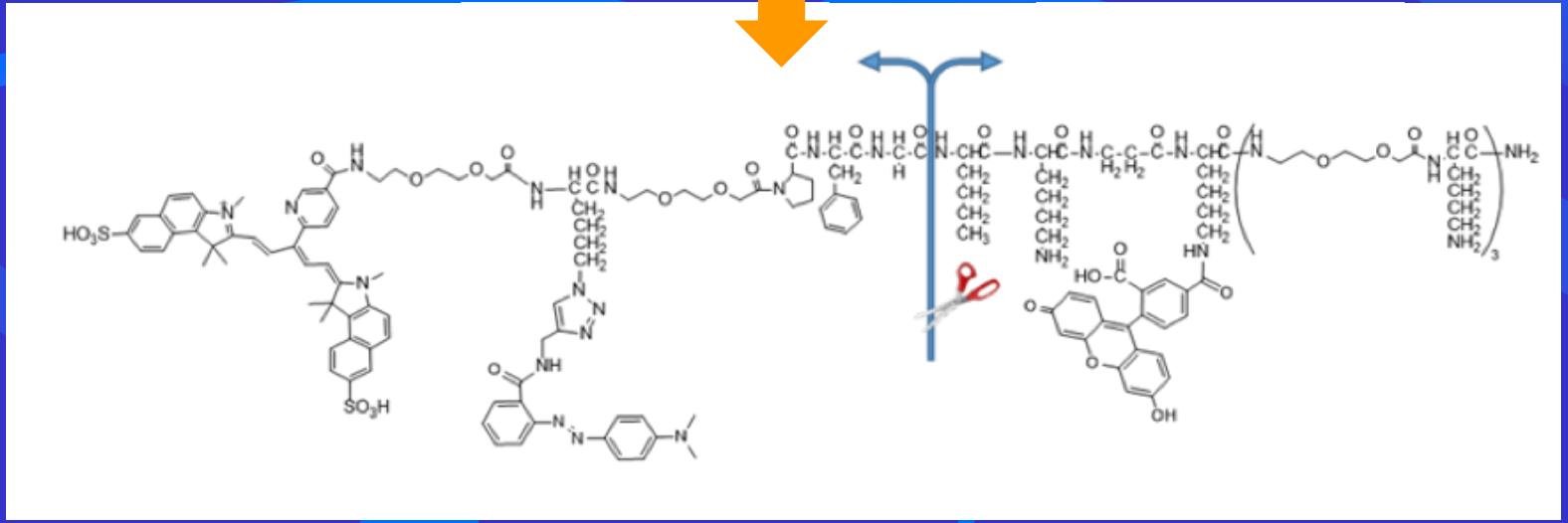


Painting Tumour Tissue

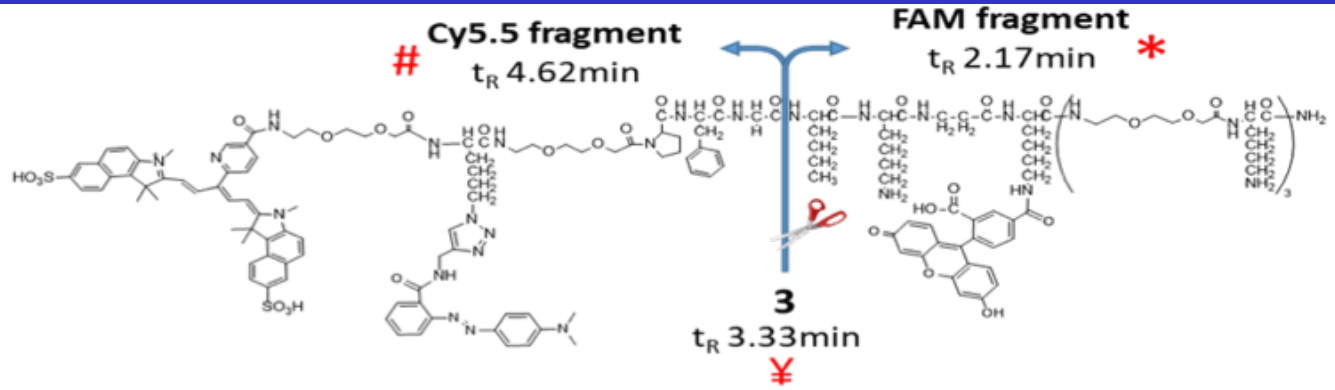


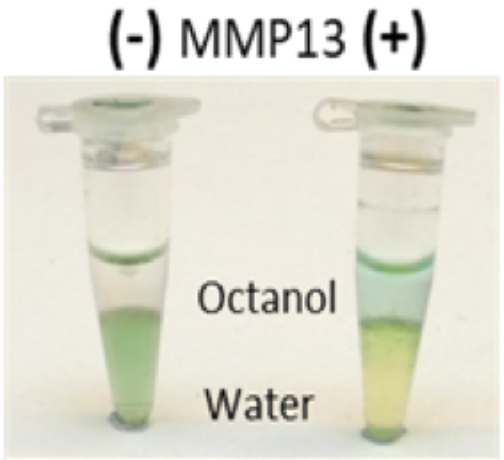
= MMPX

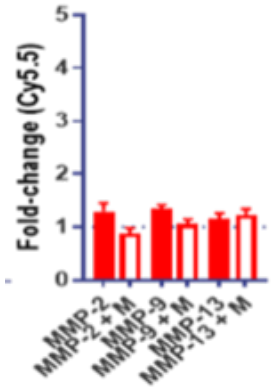
FAM-P-F-G-Nle-K-ßA-K(Quencher)-[PEG_2-k]$_2$

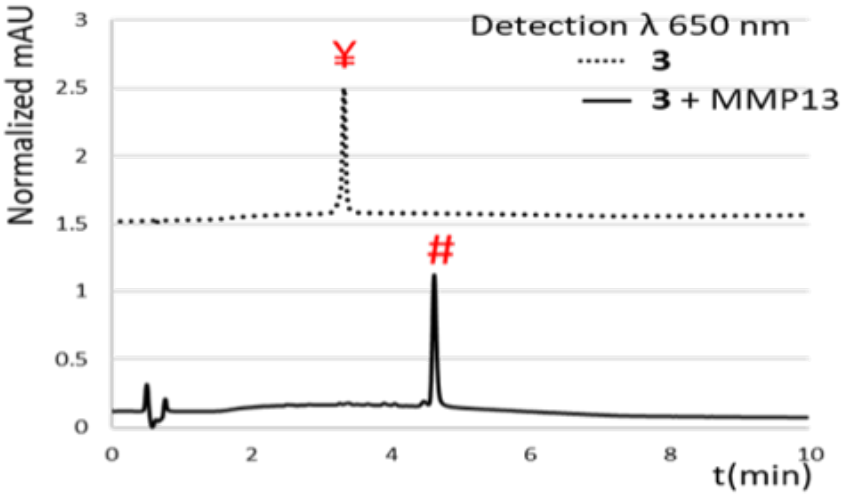


Painting Tumour Tissue

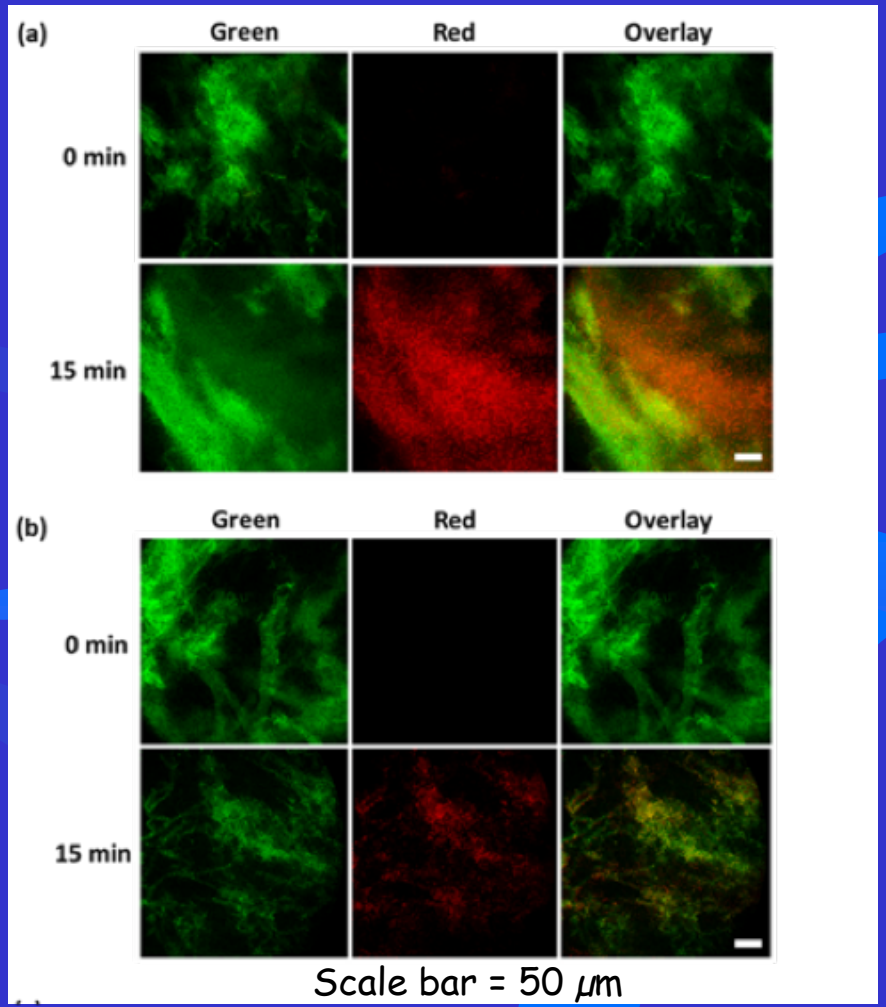








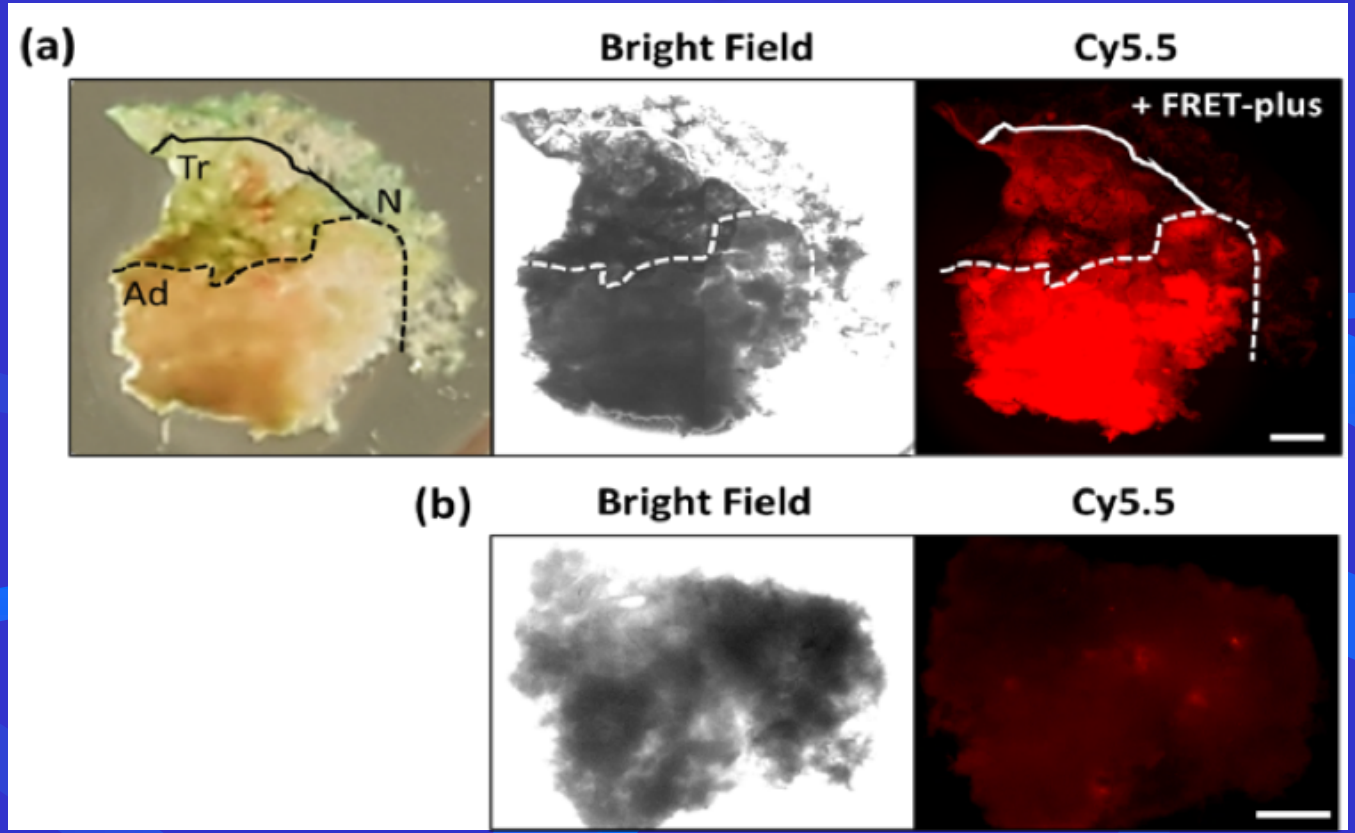
Painting Tumour Tissue



Probe (5 μM) was added to ex vivo human lung cancer tissue (+/- marimastat) and interrogated using an optical fibre-based imaging device

Frames shown from a video.

Painting Tumour Tissue



Adenocarcinoma (**Ad**), Transition zone (**Tr**) and Normal (**N**) tissue. Scale = 1 mm

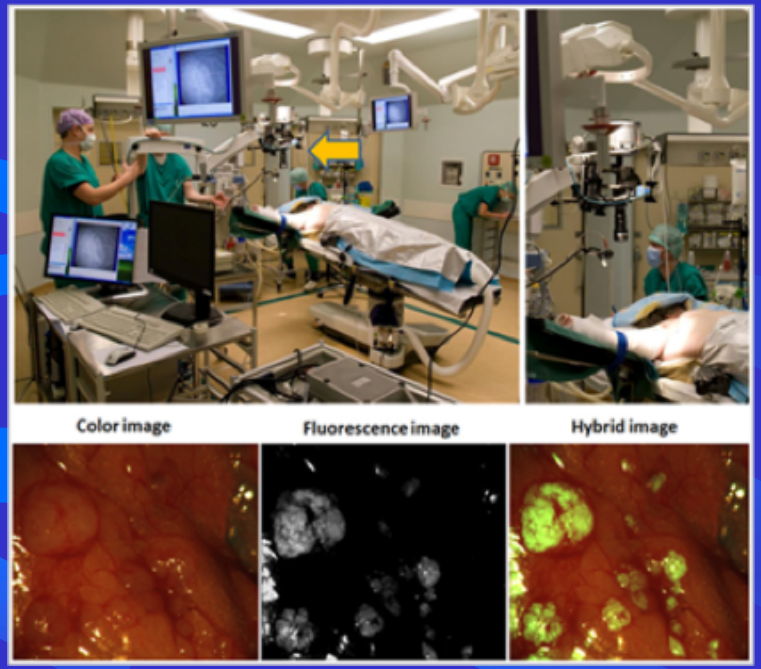
Chemical Communications 2020 (Alicia Megia-Fernandez)

MMP-Breast Cancer

- High rate of reoperation (10-30%) is a clear indication that current best practices in tumour margin demarcation are inadequate.

- **Proposition:** *"A product with a fluorescent open surgery system will assist in the detection and extent breast cancer margins for use during breast cancer surgery"*

- A real need for an affordable margin-demarcation tool that provides visual feedback intra-operatively in real time.

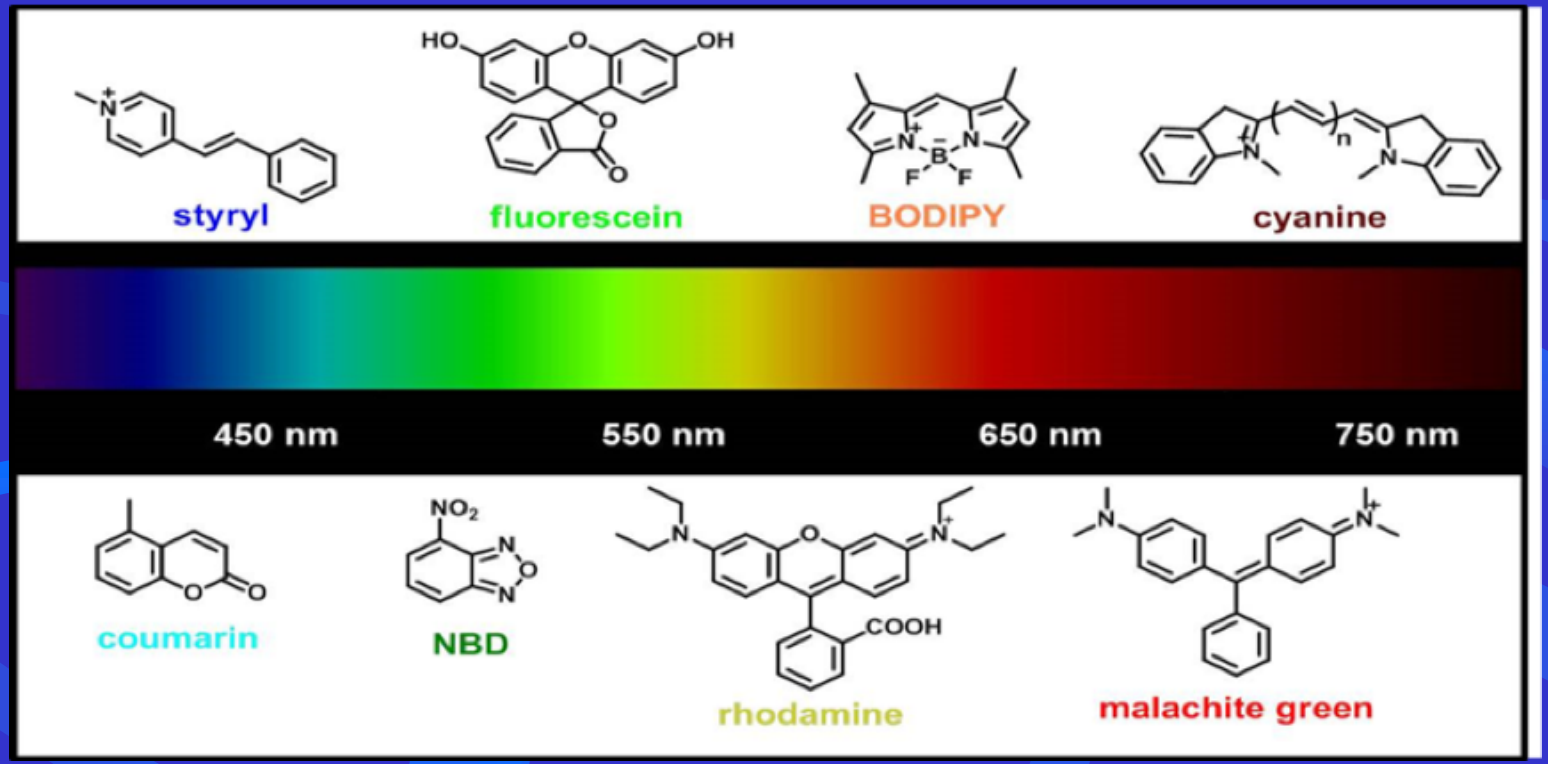


Typical Optical Surgery Facility

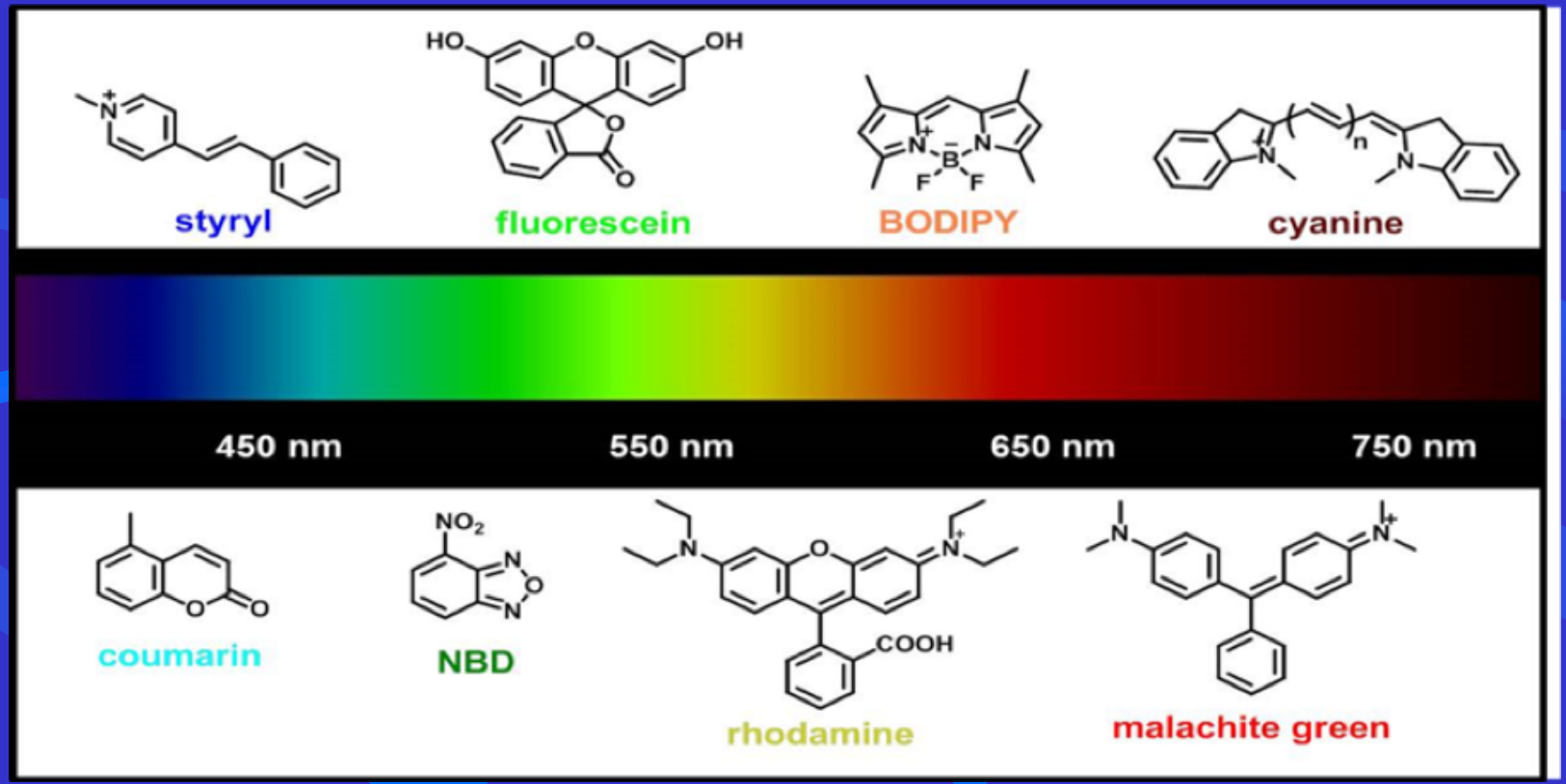
EMI-137 - Colon cancer Imaging

Proteus team has world leading detectors timing the arrival of photons (Rob Henderson)

Fluorophores – Emit Light when Excited



Fluorophores – Emit Light when Excited – BUT NOT ALL DYES EMIT AT THE SAME RATE

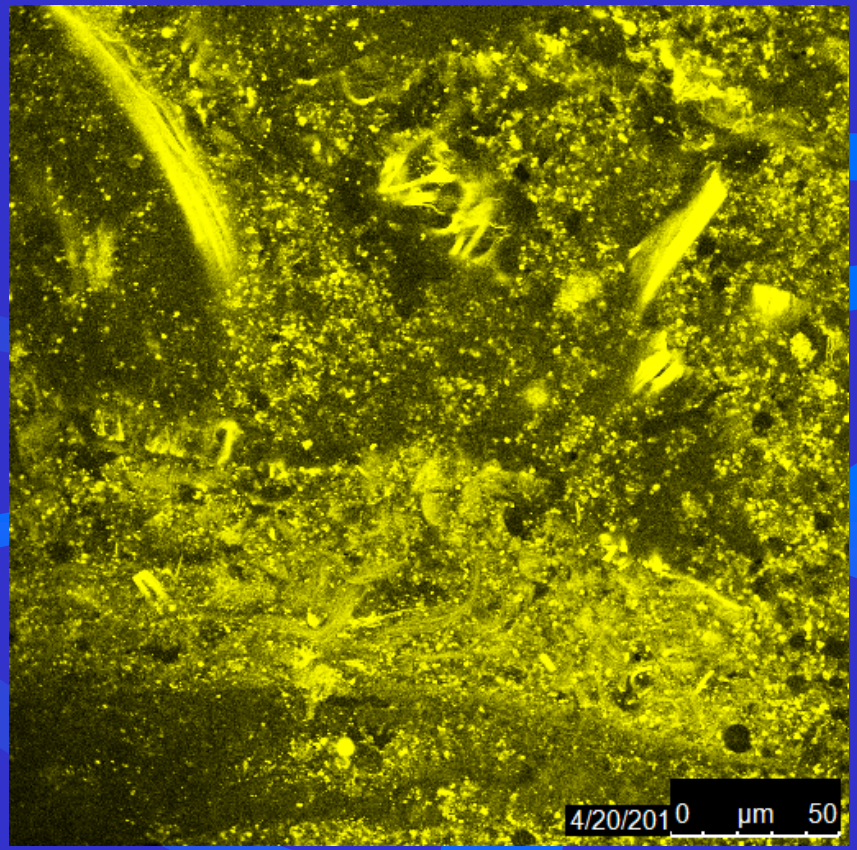


Fluorescent Life-time Analogy

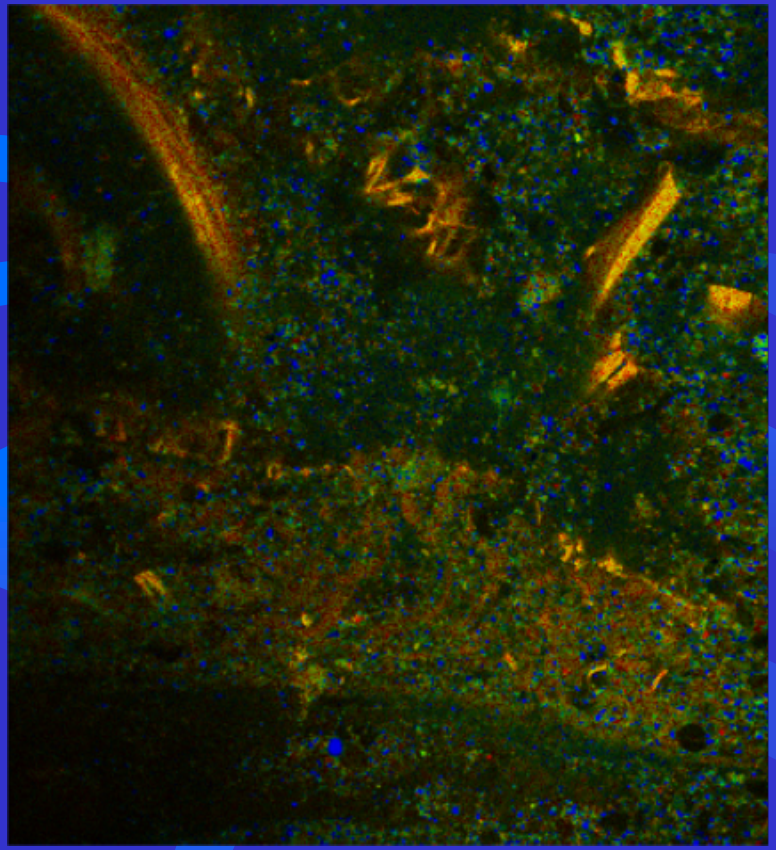
Long Fluorescent Life-time
(emits slowly)

Short Fluorescent Life-time
(emits quickly)

Is Yellow Yellow ?



Normal Microscope Image



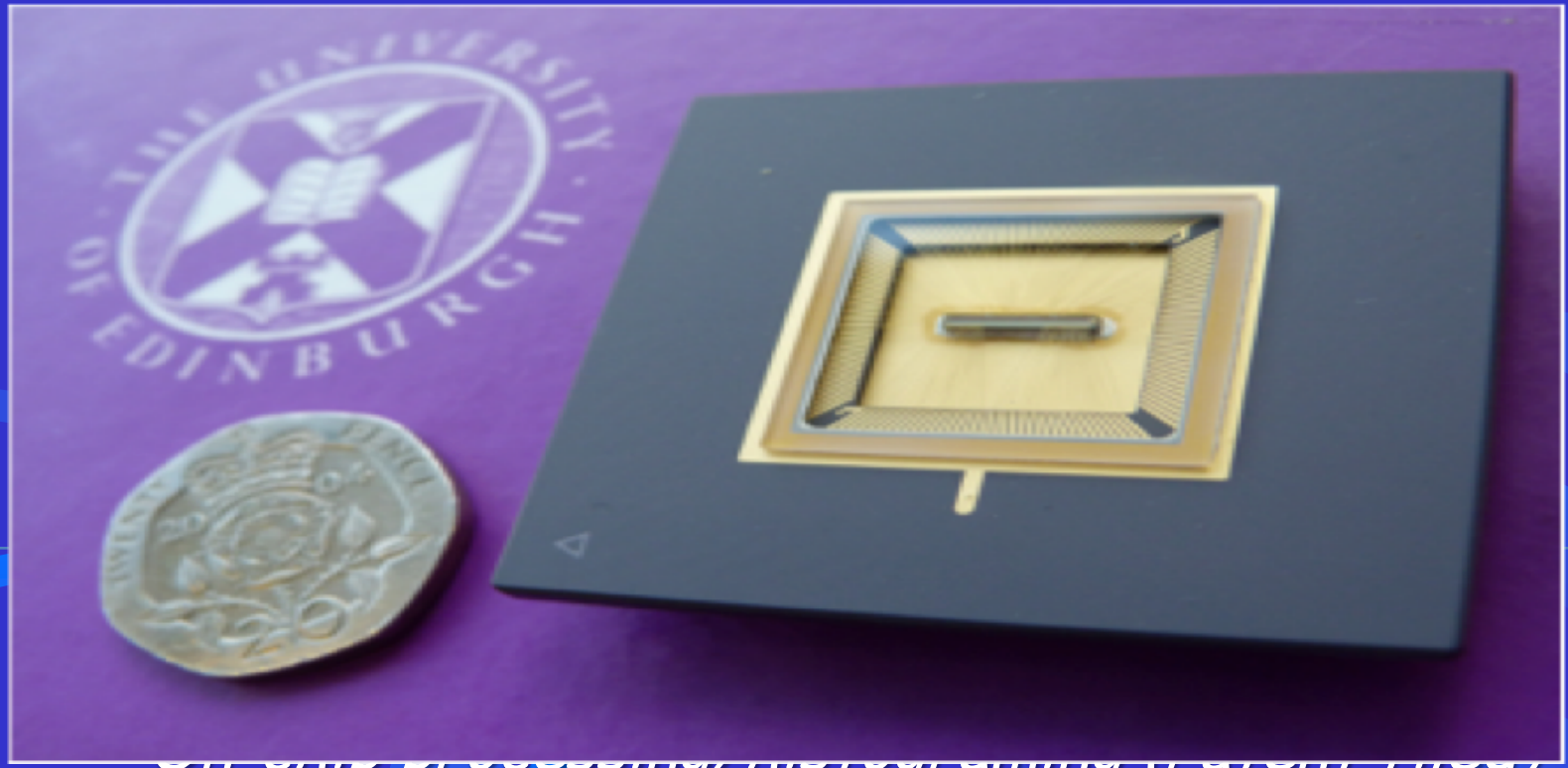
Life-time Image

Increased Contrast and Specificity: FLIM

- Fluorescence lifetime as an additional dimension for diagnostics
- Increased ability to multiplex signals
- Challenge:
  - Acquisition
  - Lifetime determination
  - Image display

Have to be fast enough to minimise motion artefacts -> high frame rate required

New Detectors - Ra-II



A 16.5 Giga Events/s 8 SPAD Line Sensor with per-pixel Zoomable 50ps-6.4ns/bin, Histogramming TDC, VLSI Symposium 2017, Kyoto, Japan, 5-8 June 2017 and N. Finlayson, R. Henderson, Time-zoomable FRET spectroscopy with a 512 x16 SPAD line sensor, Proc. SPIE 10504, Biophysics, Biology and Biophotonics (2018)

Ra-II Based Imaging System

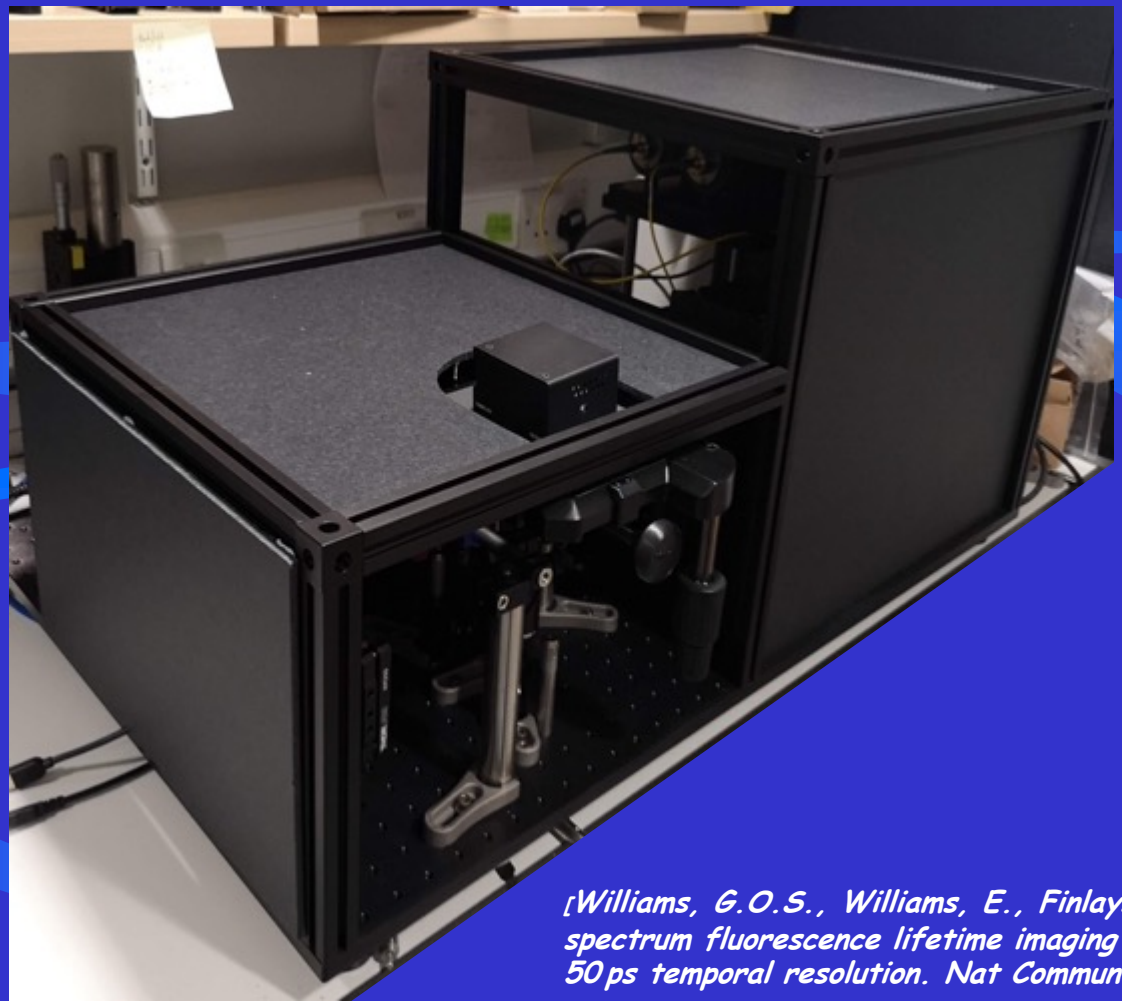
- On-chip Histogramming -> amount of data transfer greatly reduced (compared to traditional TCSPC methods)

- Photon arrival times also split by wavelength

- Collecting an image with 320x320 pixels at full temporal resolution generates: 2.15GB/s -> 0.1 Frames/s
- Pixel binning and selecting temporal resolution reduces data burden.
    - 128x128 image with 4 histogram bins
    - 88MB/s
    - 11 FPS FLIM

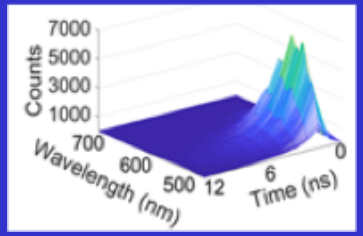
A. T. Erdogan et al, 2017 Symposium on VLSI Circuits Digest of Technical Papers
A. T. Erdogan et al, Proc. of SPIE Vol. 10504 105040M1-9

Lifetime Imaging System

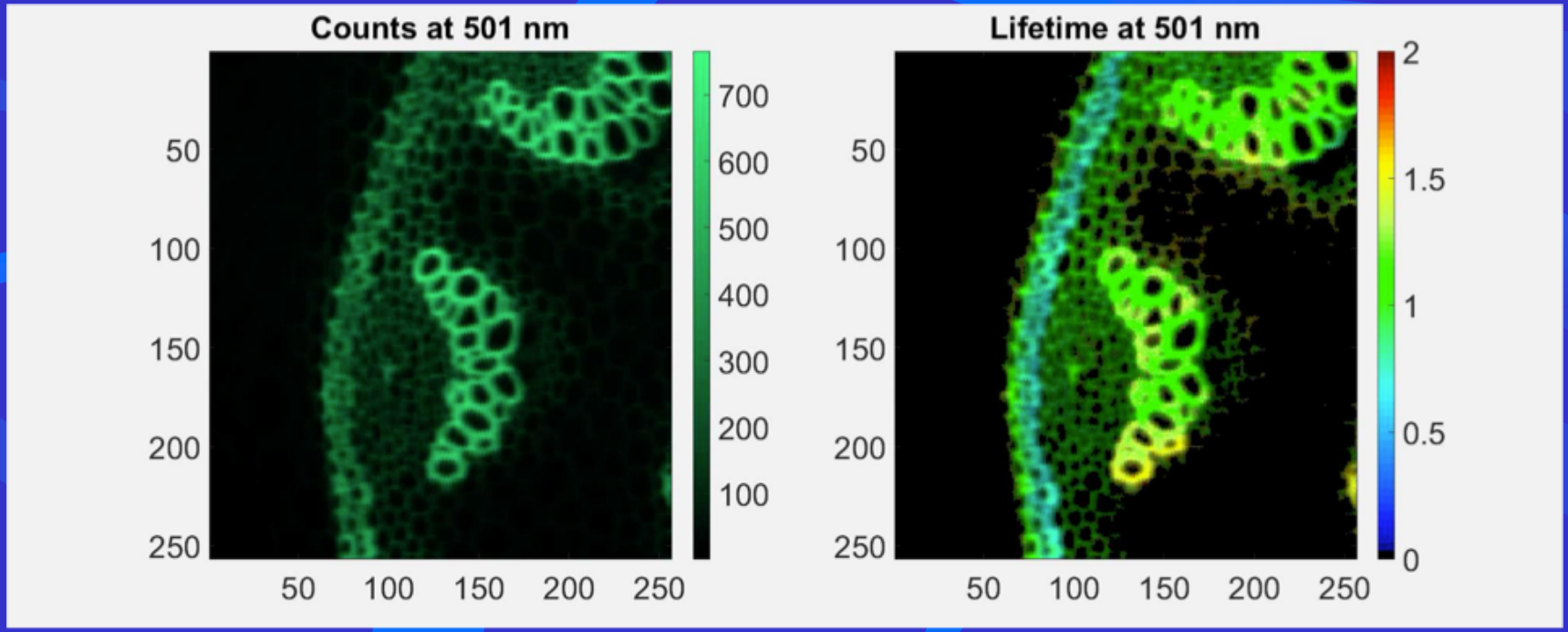


[Williams, G.O.S., Williams, E., Finlayson, N. et al. Full spectrum fluorescence lifetime imaging with 0.5nm spectral and 50ps temporal resolution. Nat Commun 12, 6616 (2021).]]

Full Exploitation of Ra-II

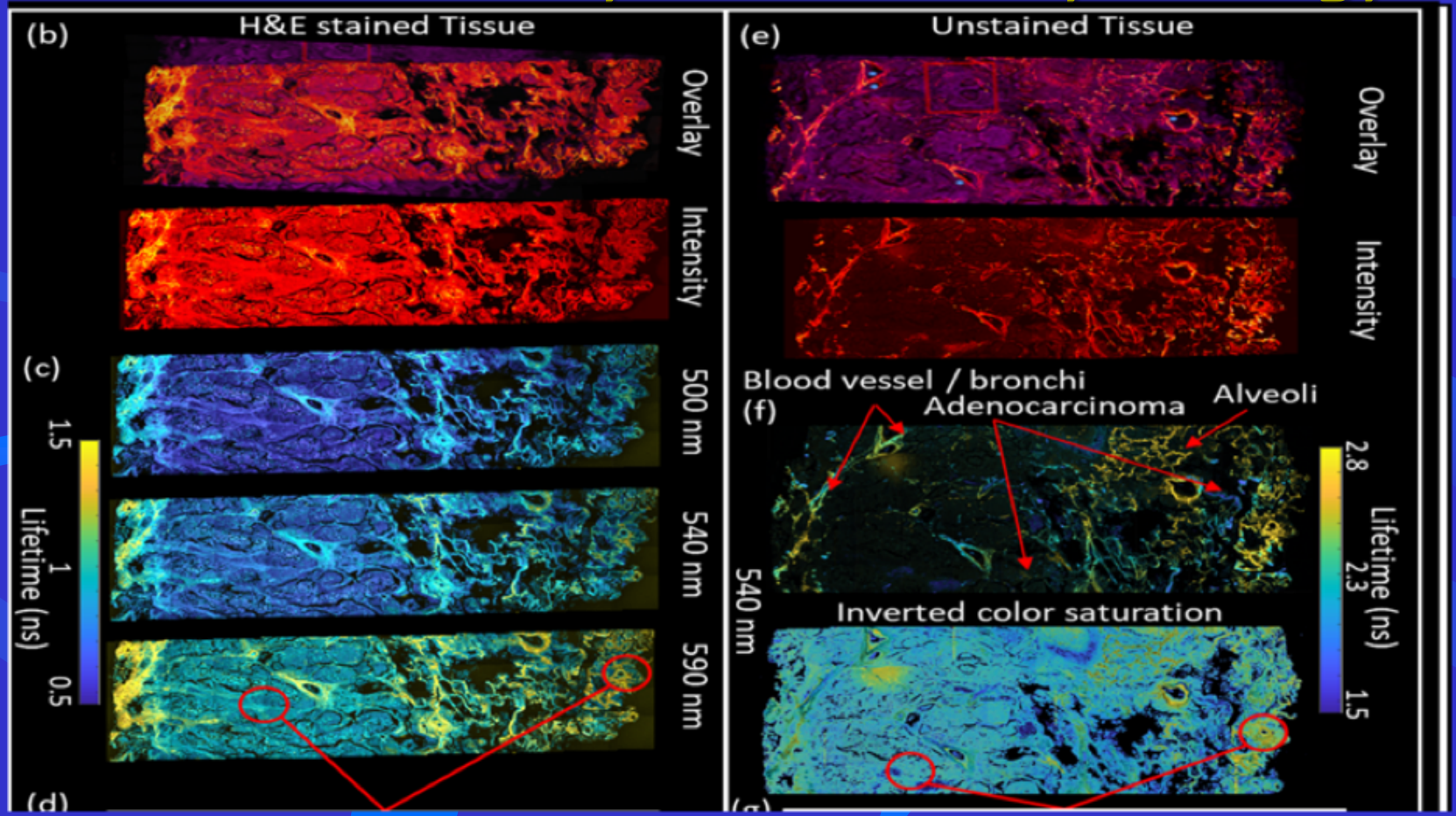


Inverted confocal microscope - for full-spectral FLIM of diverse samples

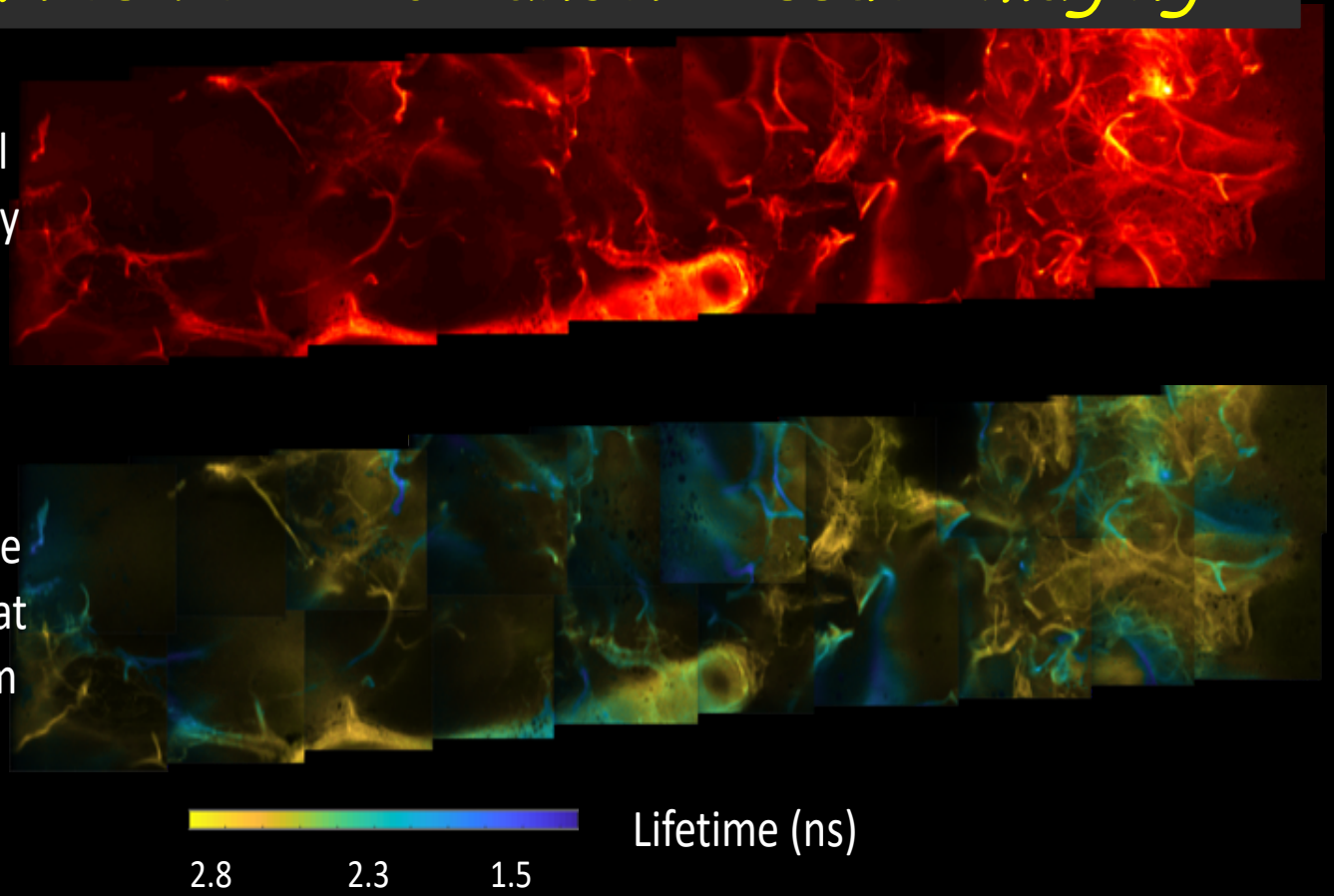


[Williams, G.O.S., Williams, E., Finlayson, N. et al. Full spectrum fluorescence lifetime imaging with 0.5 nm spectral and 50 ps temporal resolution. Nat Commun 12, 6616 (2021).]]

Potential of Full Spectral Imaging in Areas such as Spectral Histopathology



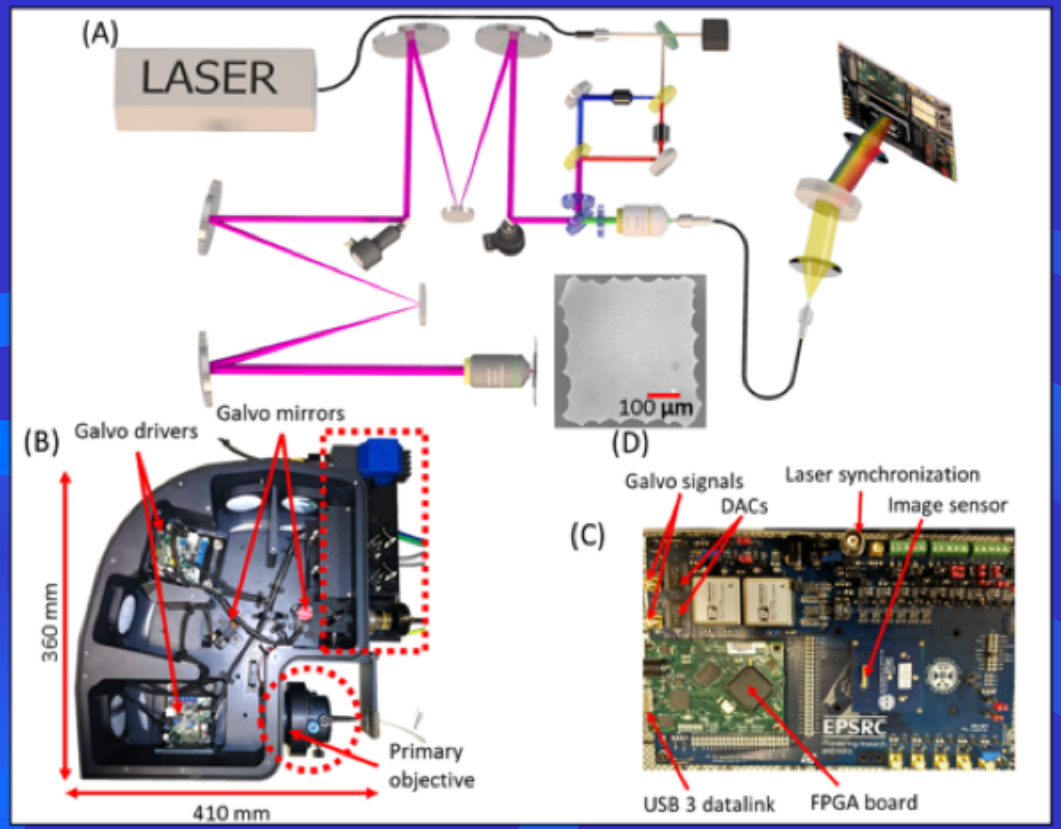
Fresh ex vivo Cancer Tissue Imaging



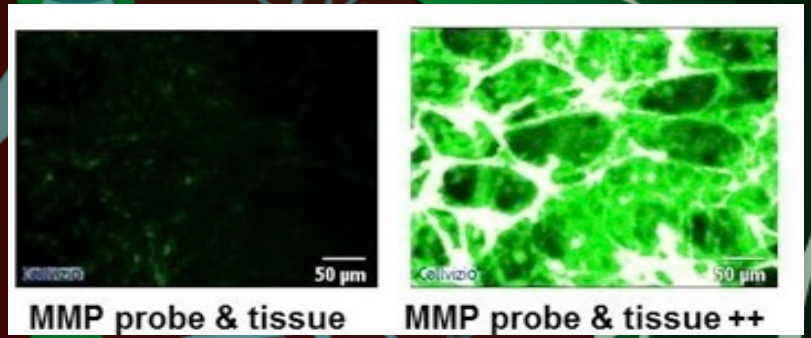
[Williams, G.O.S., Williams, E., Finlayson, N. et al. Full spectrum fluorescence lifetime imaging with 0.5 nm spectral and 50 ps temporal resolution. Nat Commun 12, 6616 (2021).]]

Compact Optical System

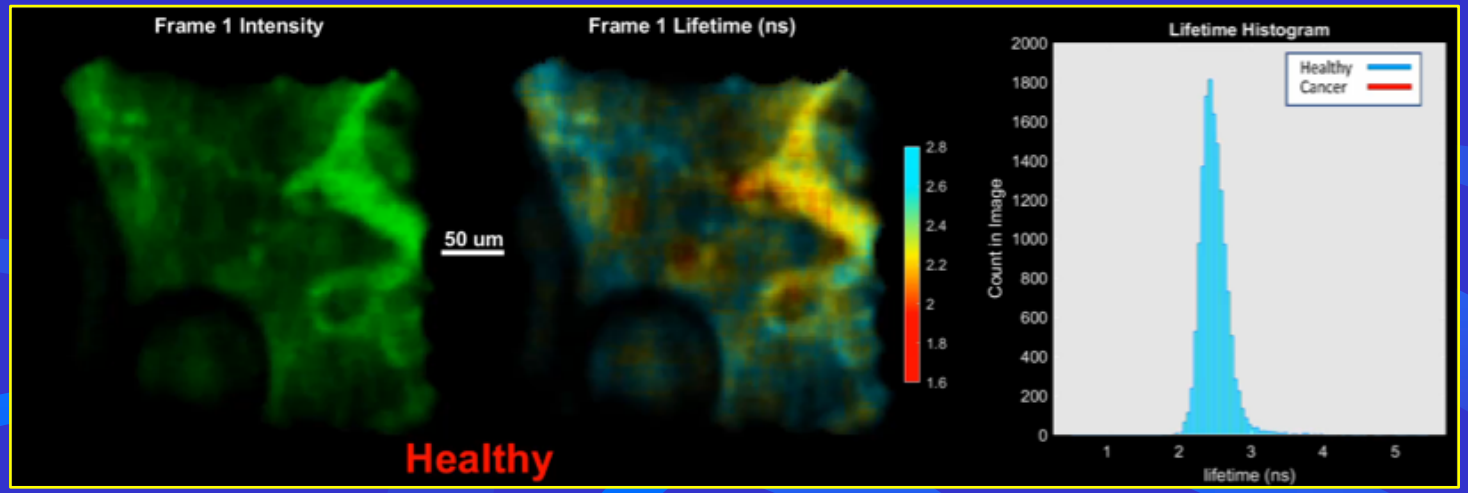
NKT Photonics
Supercontinuum



Our Approach - Optical Molecular Biopsy in vivo in situ



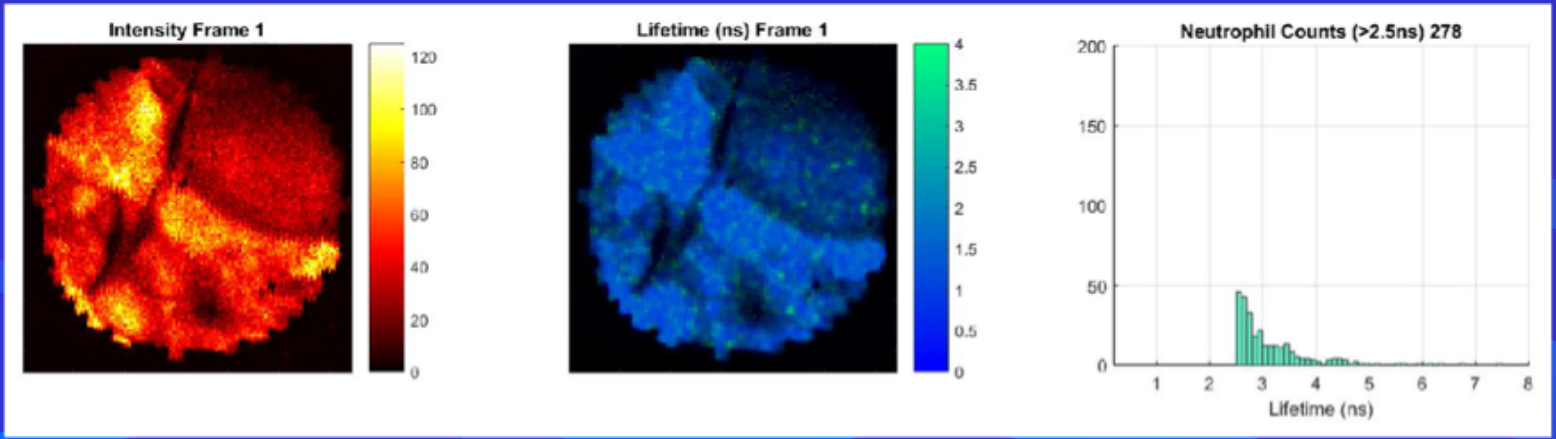
Scanning Lung Cancer



Ex vivo freshly resected specimens of healthy and cancerous tissue from sample patient.

Gareth Williams et. al. Full spectrum fluorescence lifetime imaging with 0.5 nm spectral and 50 ps temporal resolution. Nat Commun 12, 6616 (2021).

Visualising with Fluorescently Labeled Chemical Probes and Extrinsic Fluorophores



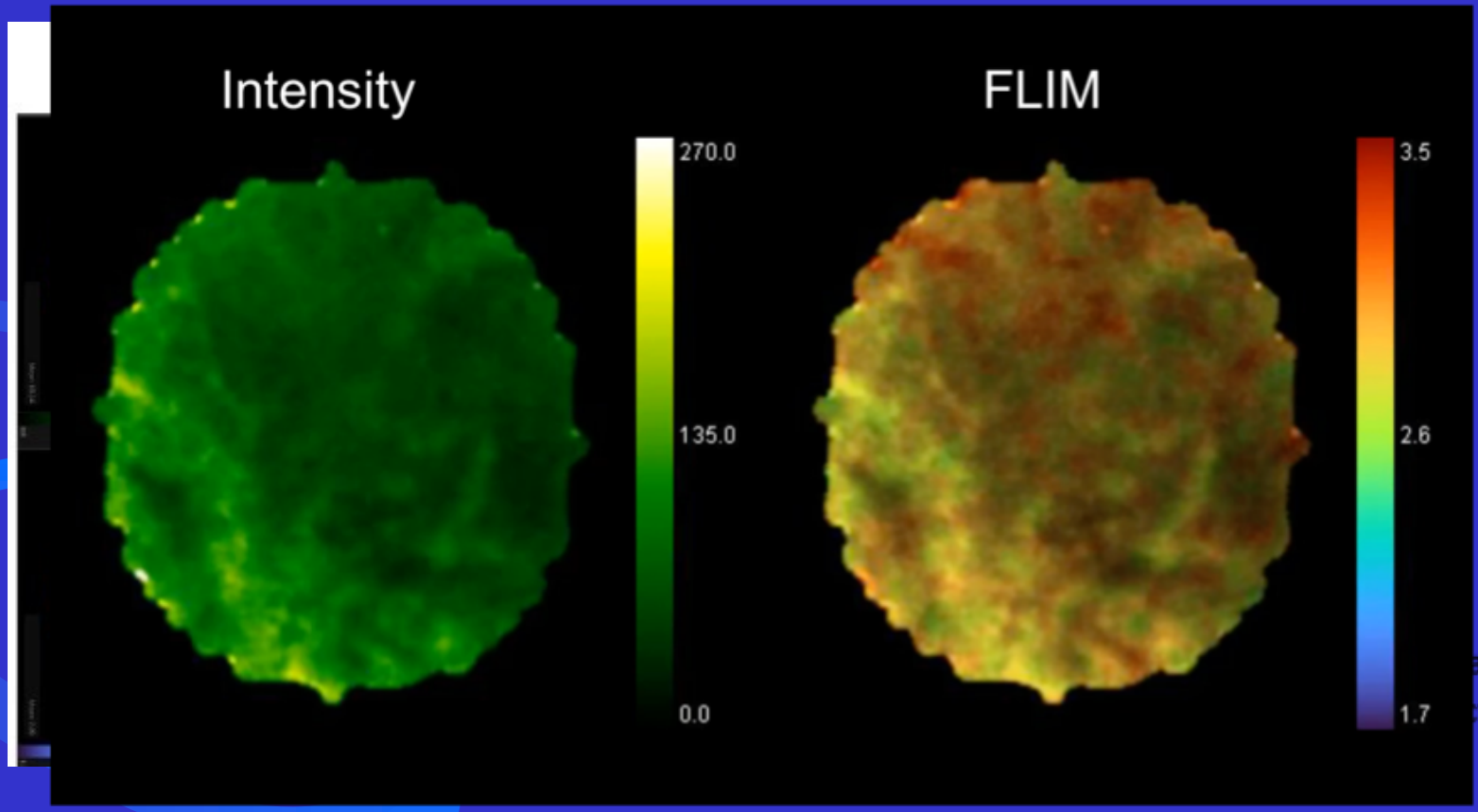
- *Perfused, ventilated human lung with Neutrophil Activation Probe.*
- *Detecting pulmonary neutrophil recruitment during Acute Respiratory Distress Syndrome.*

Fluorescent Probe for FAP (Fibroblast Activation Protein)

- Upregulation of Fibroblast Activation Protein (FAP) associated with cancer and chronic inflammation.
- A library of compounds was synthesised to develop an optical probe to monitor FAP activity in fibroblasts
- D-Amino acid incorporation provides a means of generating good specificity (DDP-IV and various MMP's).

Alicia Megia-Fernandez, Ahsan Akram *Front. Oncol.* **2022**, 12:834350

ex vivo Detection of FAP – KronoScan and Lifetime Imaging



Ex vivo lung tissue imaging

Alicia Megia-Fernandez, Ahsan Akram *Front. Oncol.* **2022**, 12:834350

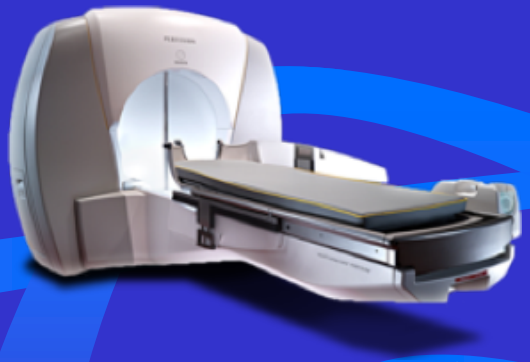
Tumor Imaging – Optical Probes and Fluorescent Lifetime

- Fluorescent probes used for in vivo imaging and drug engagement studies
- Probes designed to light up tissue with specific protease activity
- Fluorescent life-time capability allows cancer margin detection :
  - Targeting use during surgery
  - Chip-on-tip life-time imaging sensor on-going (Endocan – 1.5 mm x 1.5 mm)

Radiotherapy Activation of Prodrugs

Nature Chemistry 2021

γ-Ray Triggered Prodrug Activation

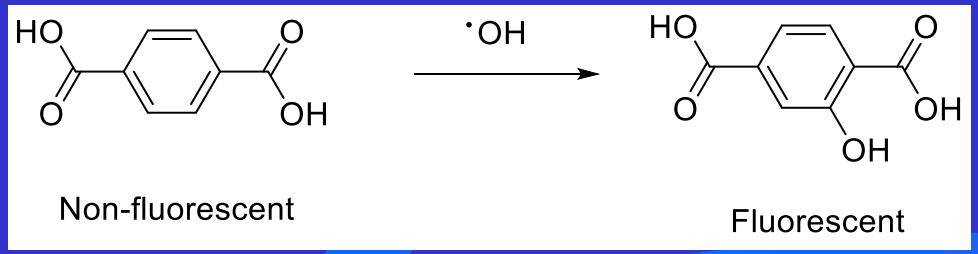


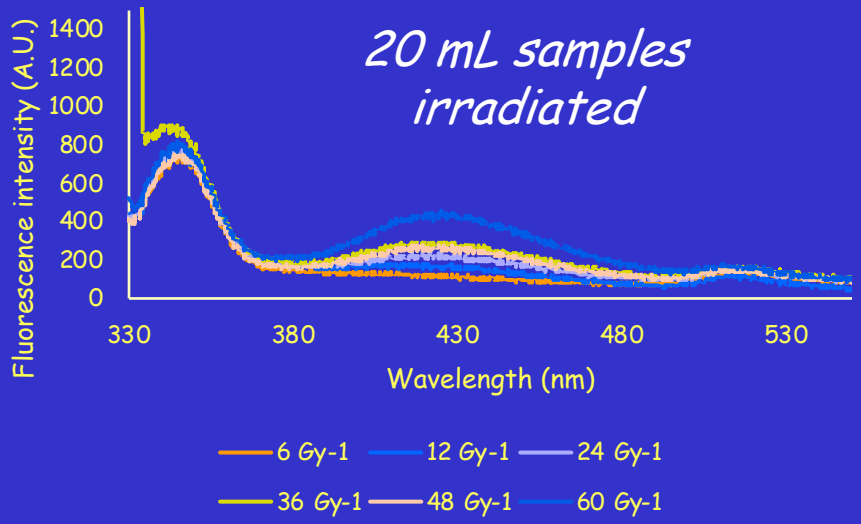
Prodrug → Active Drug

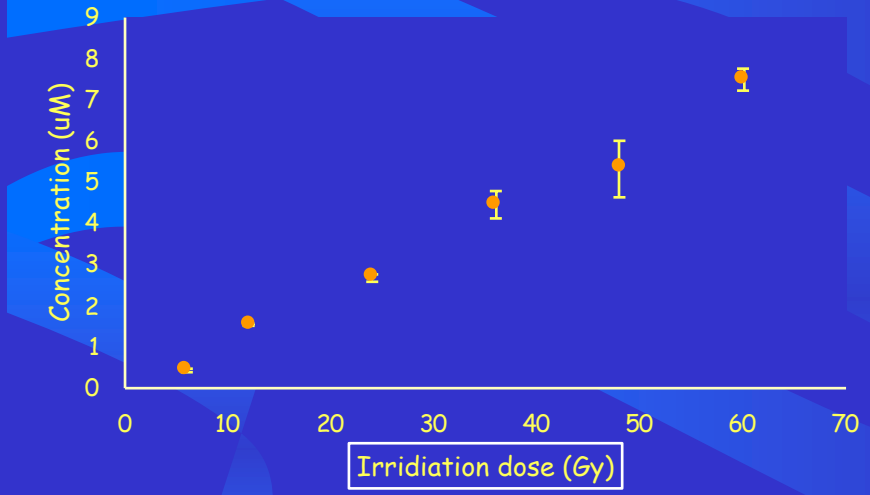
Gamma or X-Ray

- Selective and controlled activation within the tumour
- Dual treatment effect (chemotherapy and radiology)
- Other possible additional "killing" processes

Hydroxy Radical Generation





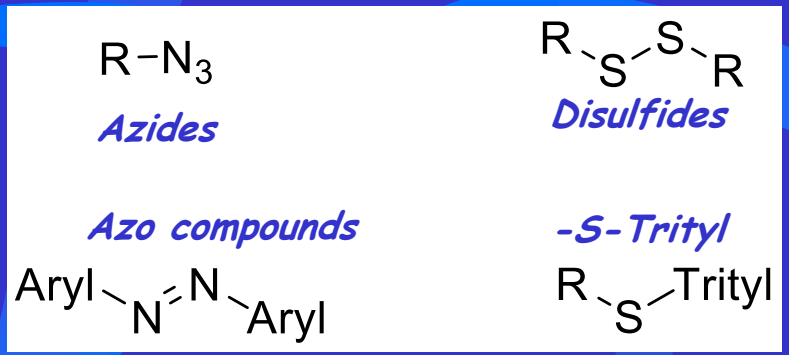


20 mLs of 8 μM compound with 60 Gy

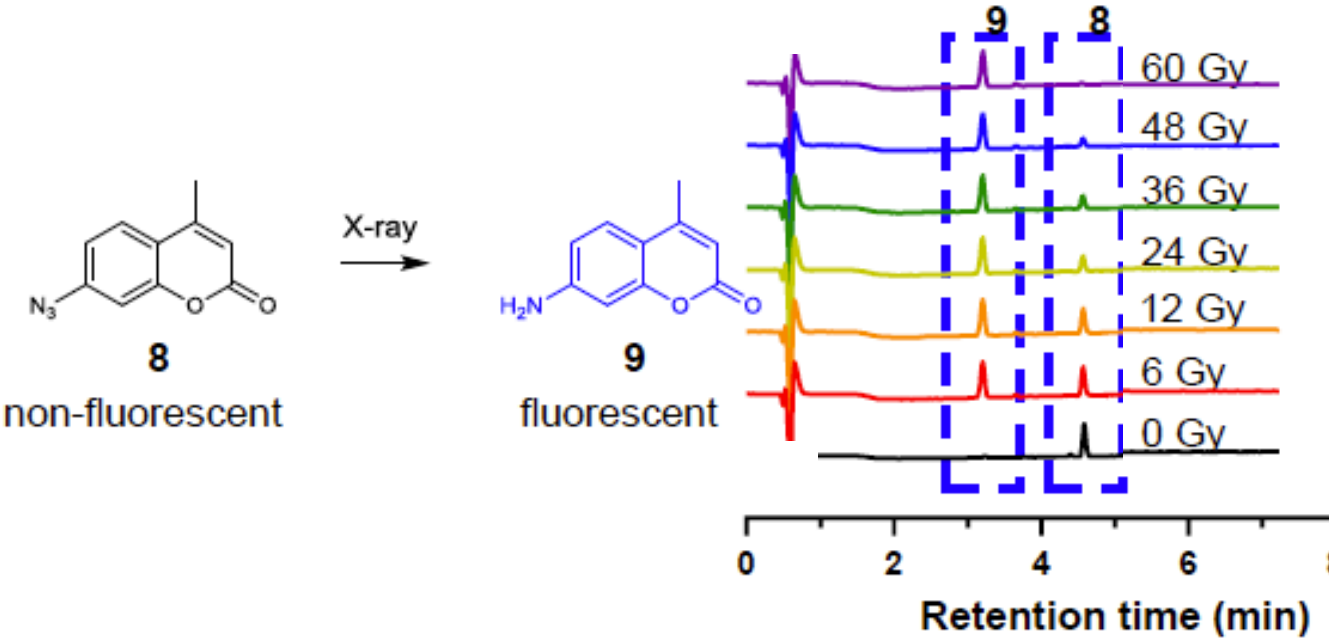
High-throughput γ-Ray Screening of Chemical Moieties

- *Goal: Identification of γ-ray sensitive chemical moieties*
- *Procedure: Irradiation (100 Gy) of samples (200 µM) in degassed PBS/MeOH (1/1)*

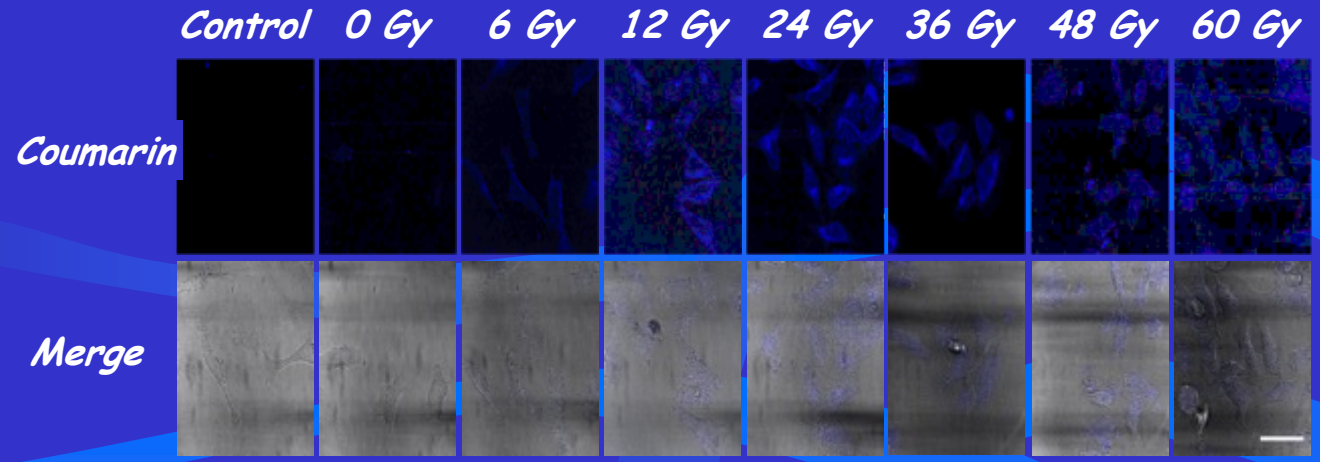
- *Four γ-ray sensitive functional groups were identified:*

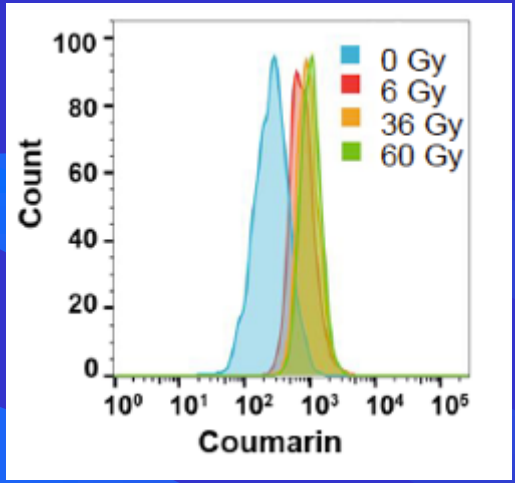


Coumarin Azide Activation and Cell Labelling

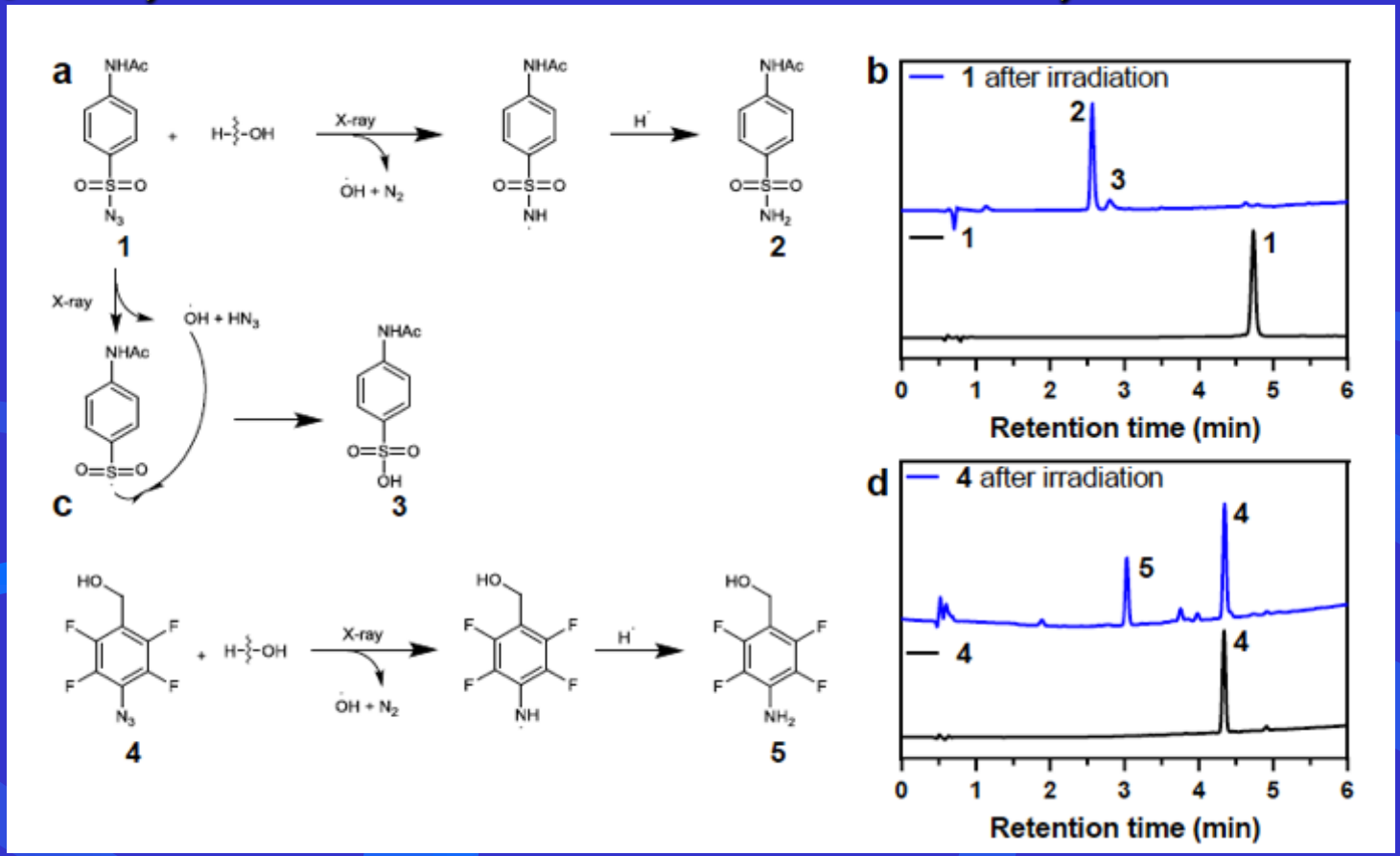


Coumarin Azide Activation in Live Cells via γ-Ray Irradiation





γ-Ray Mediated Azide Chemistry - Reduction

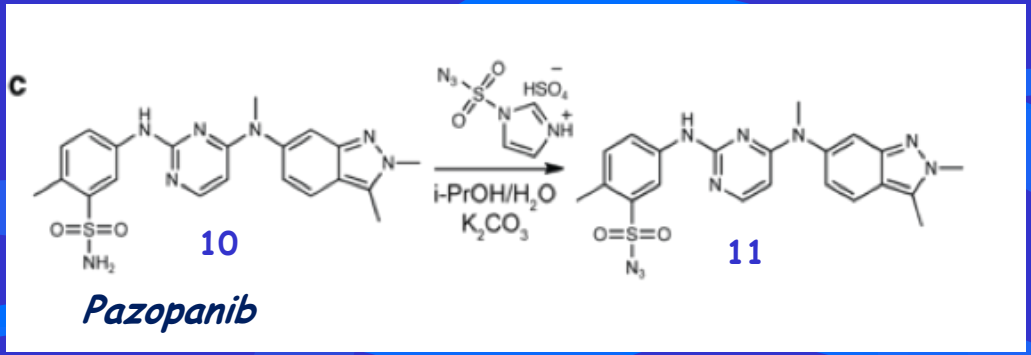


Via a sulfonylamido radical or a nitrene (The photolysis of sulfonyl azides in isopropyl alcohol, JACS 1964, A.Nickon that describes the radical mediated generation of sulfonamides from sufonyl azides)

γ-Ray Screening of Chemical Moieties

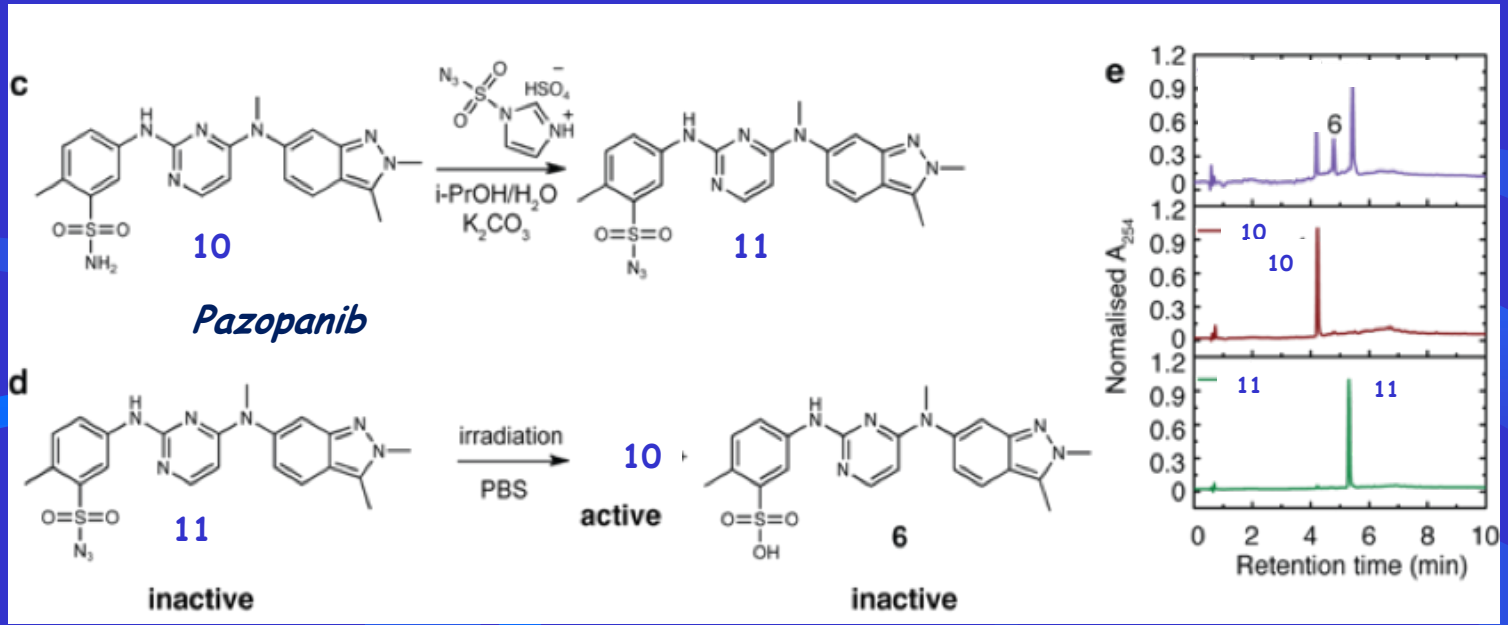
- ✓ Identification of chemical moieties that respond to γ- rays
- ✓ Azides and azo compounds are bio-inert and a common prodrug scaffold
- ✓ Reaction takes place at low micro molar concentrations
- ✓ Synthesis of many azide or azo protected drugs possible !

Radiotherapy Activation of Prodrugs



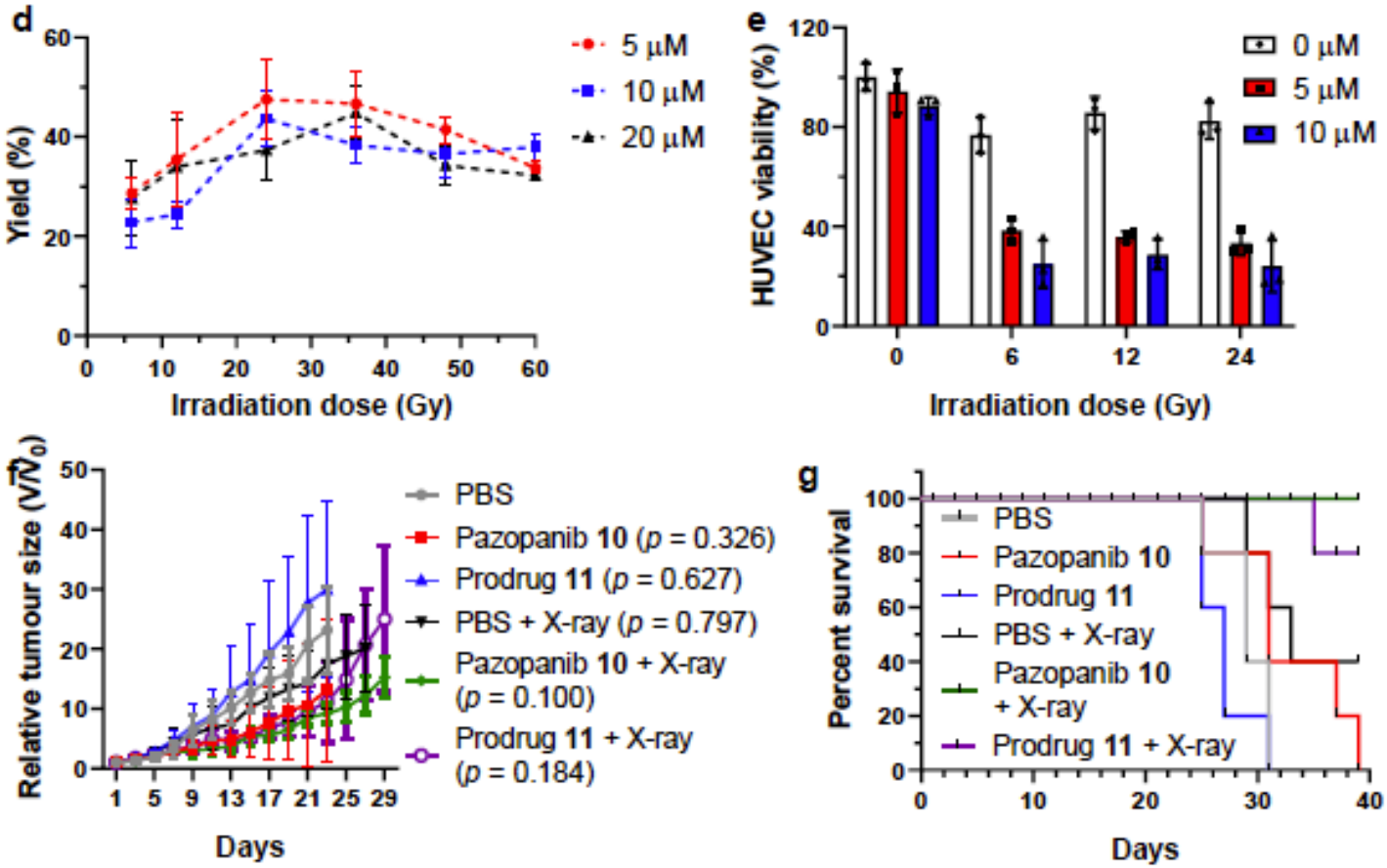
Pazopanib (Votrient) is a potent receptor tyrosine kinase inhibitor used clinically for renal cell carcinoma and soft tissue sarcoma

Radiotherapy Activation of Prodrugs

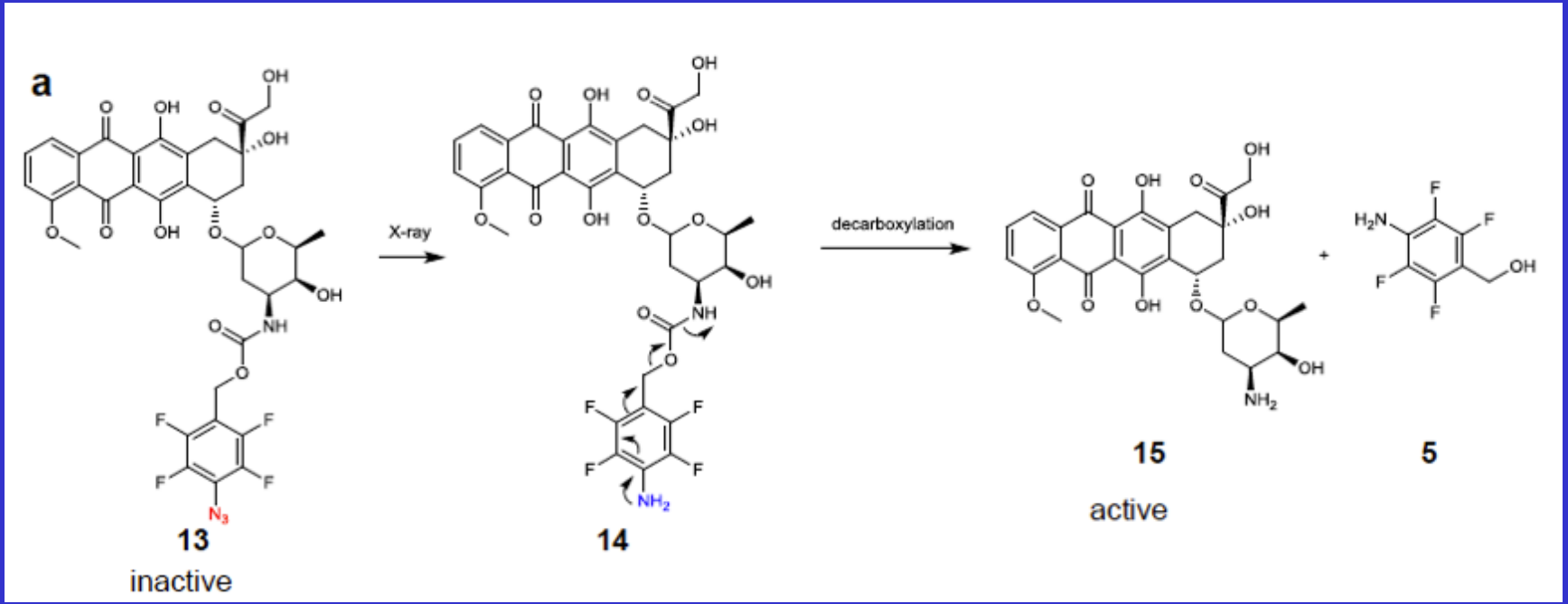


Pazopanib = Pazopanib (Votrient) is a potent receptor tyrosine kinase inhibitor used clinically for renal cell carcinoma and soft tissue sarcoma

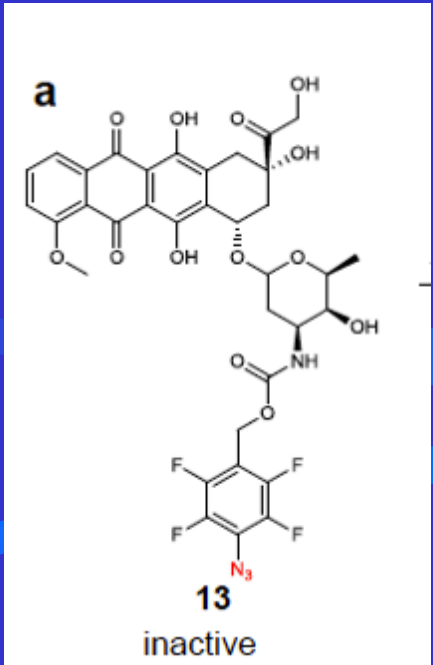
Radiotherapy Activation of Prodrugs



Doxorubicin-Prodrug Azide Activation

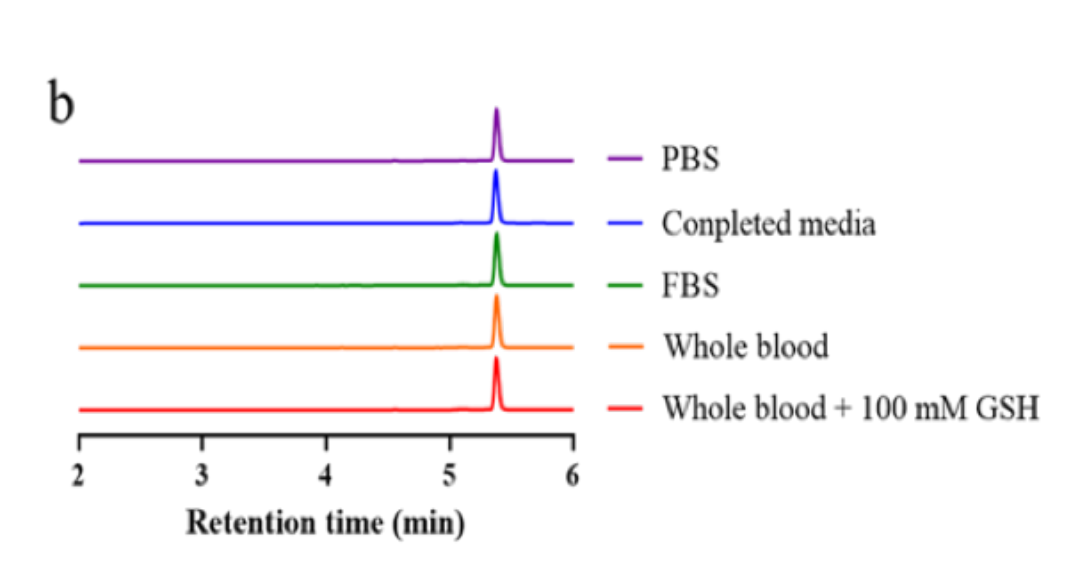


Azide Stability in vivo

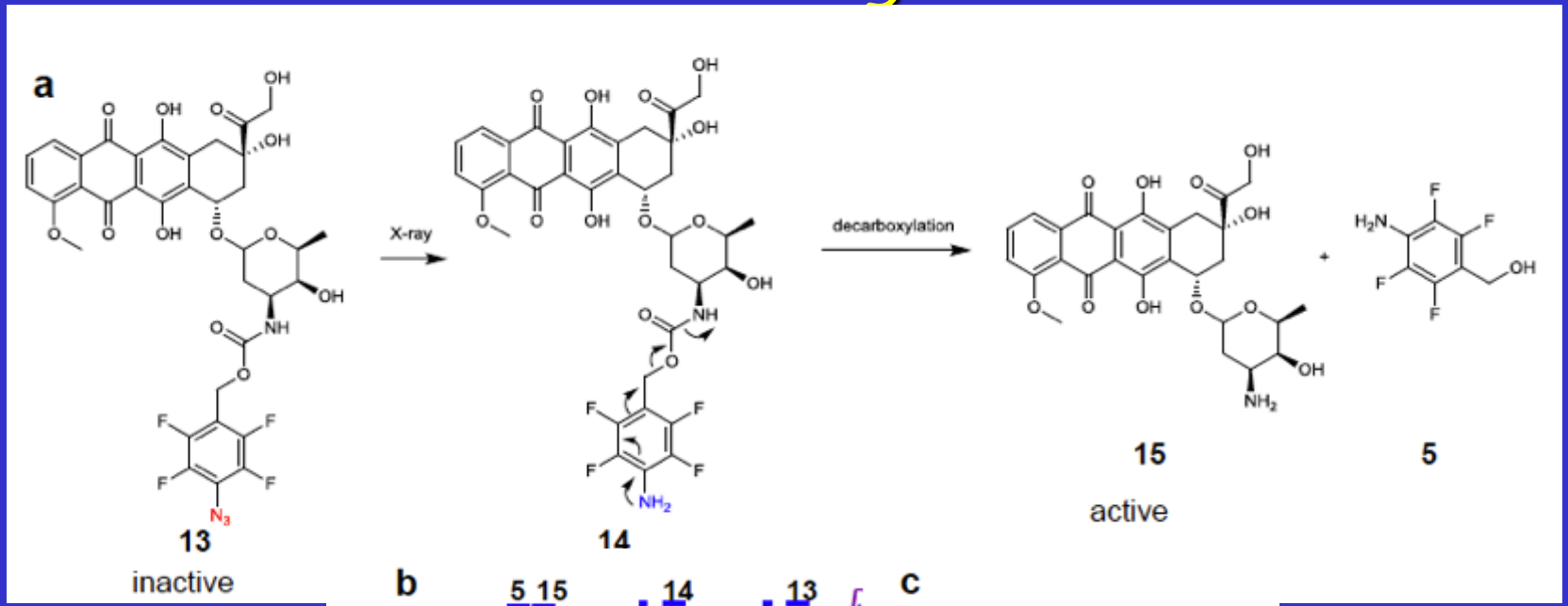


13

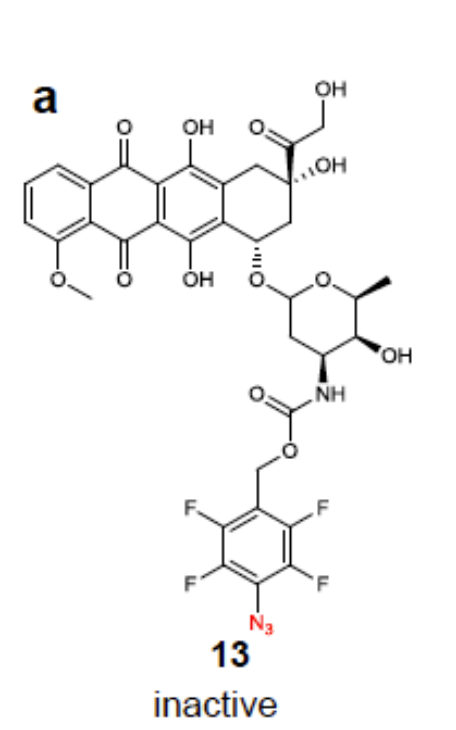
inactive

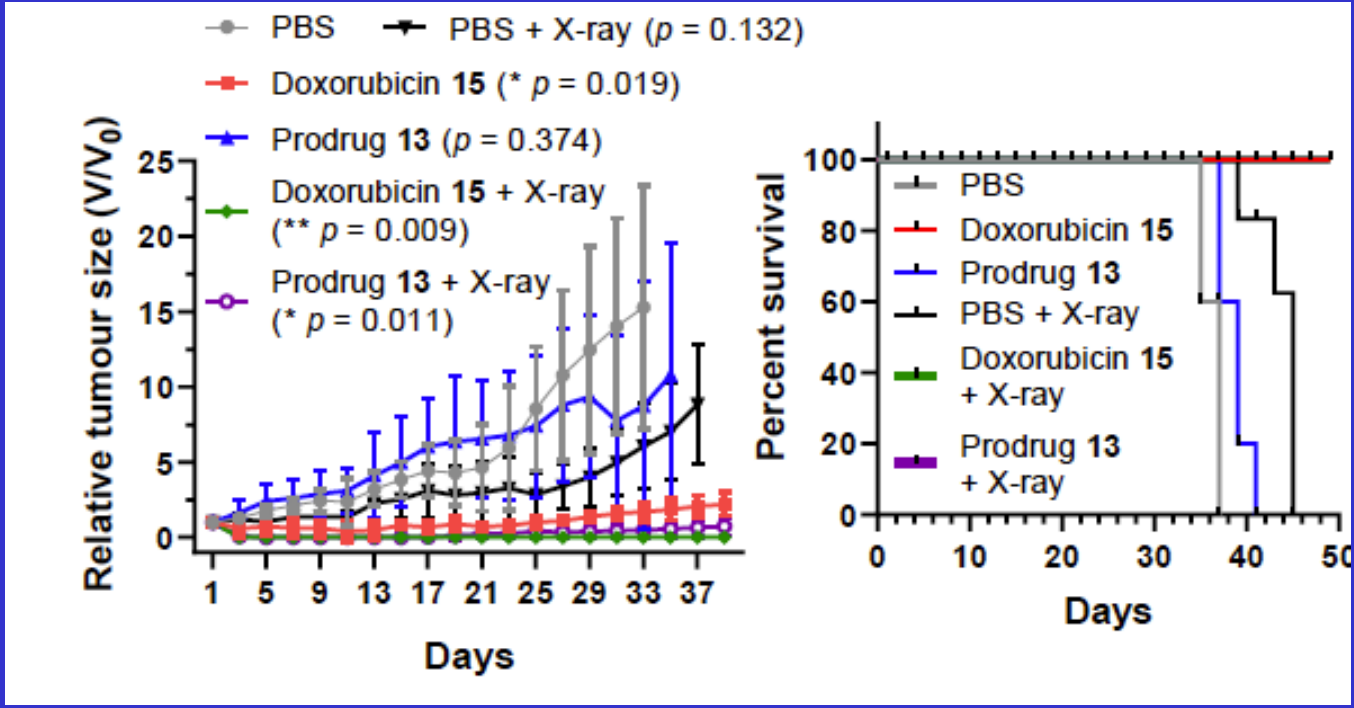


Doxorubicin-Prodrug Azide Activation



Doxorubicin-Prodrug Azide Activation – in vivo studies





Radiotherapy Activation of Prodrugs

- Radiotherapy can be used to active prodrugs in vivo
- Generates 10μM of compound within a volume of 20 mLs.
- Generic "azido-tetrafluoro-aryl group" allows drugs with amines or hydroxy groups to be "pro-drugged".
- Now targeting antibody drug conjugate linkages.
- Exploration of other linkages underway.

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Professor Mark Potter

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