ACTIVATABLE FLUORESCENT PROBES FOR LIVE-CELL IMAGING

Marc Vendrell
marc.vendrell@ed.ac.uk
www.dynafluors.co.uk

OUTLINE

- Optical transduction
- Basic concepts in fluorescence imaging
- Common optical techniques used in chembio labs
- Chemical development of fluorescent probes
- Case-studies of fluorescent probes used in cell imaging

OPTICAL TRANSDUCTION MECHANISMS

Most common optical properties used in cell-based assays:

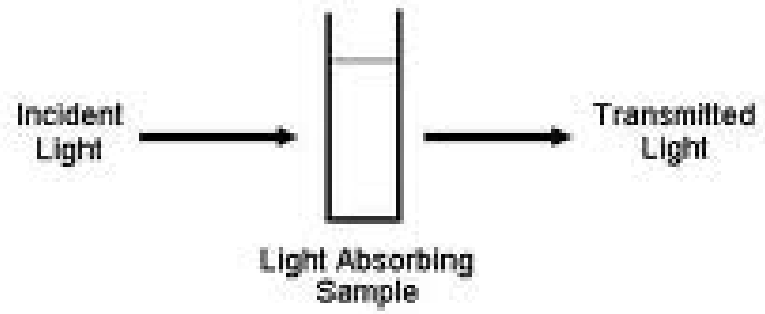
- absorbance

- fluorescence

- chemi- or bioluminescence

OPTICAL TRANSDUCTION MECHANISMS

Absorbance (A): radiation transmitted through a material.



$A = -\log (I_1/I_0)$

Beer's Law : $A = \varepsilon c \ell$

Where:

A = absorbance or Optical Density

ε = extinction coefficient of absorbing substance

c = concentration of light absorbing substance

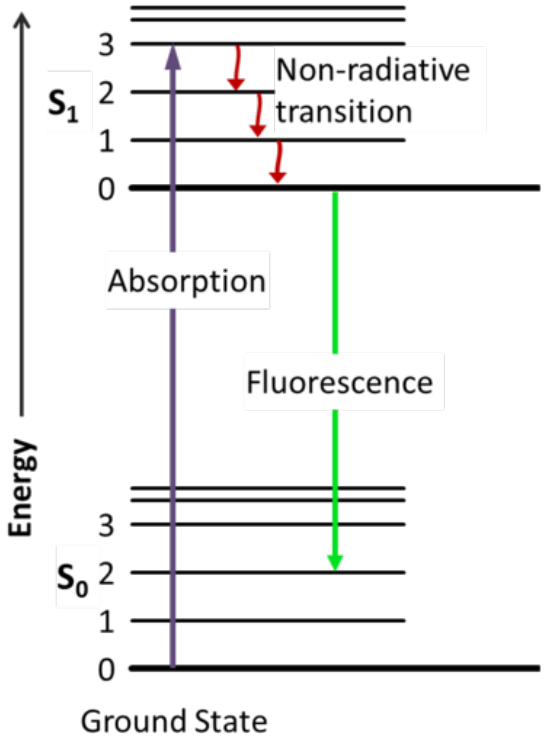
ℓ = pathlength of the light absorbing substance

.

Simple, cheap, potential interference from sample, not always is very sensitive.

OPTICAL TRANSDUCTION MECHANISMS

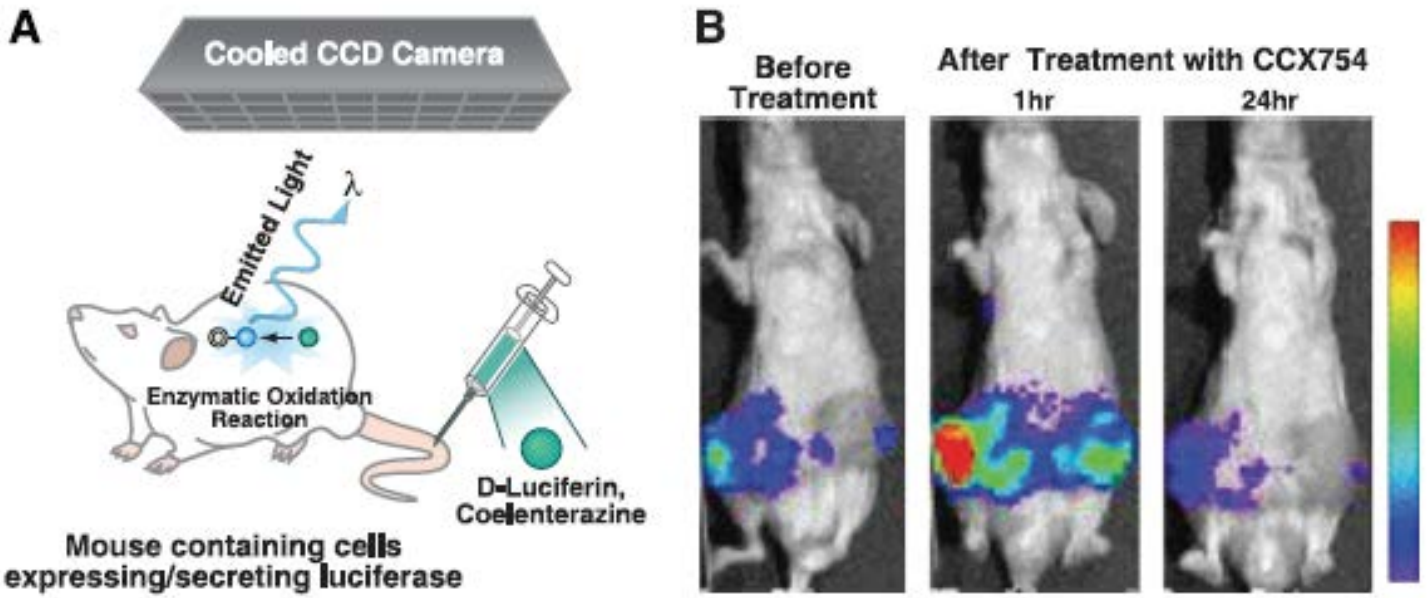
Fluorescence: emission of light upon irradiation at a specific wavelength.



Highly sensitive, still cheap, less interference from cells. Probes available.

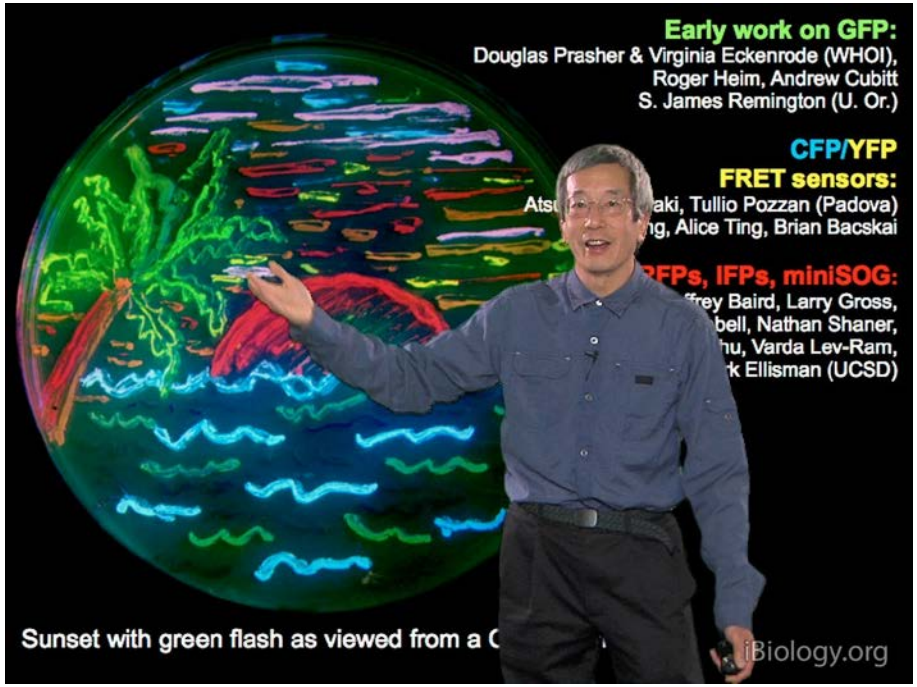
OPTICAL TRANSDUCTION MECHANISMS

Chemi/bioluminescence: emission of light upon a chemical reaction

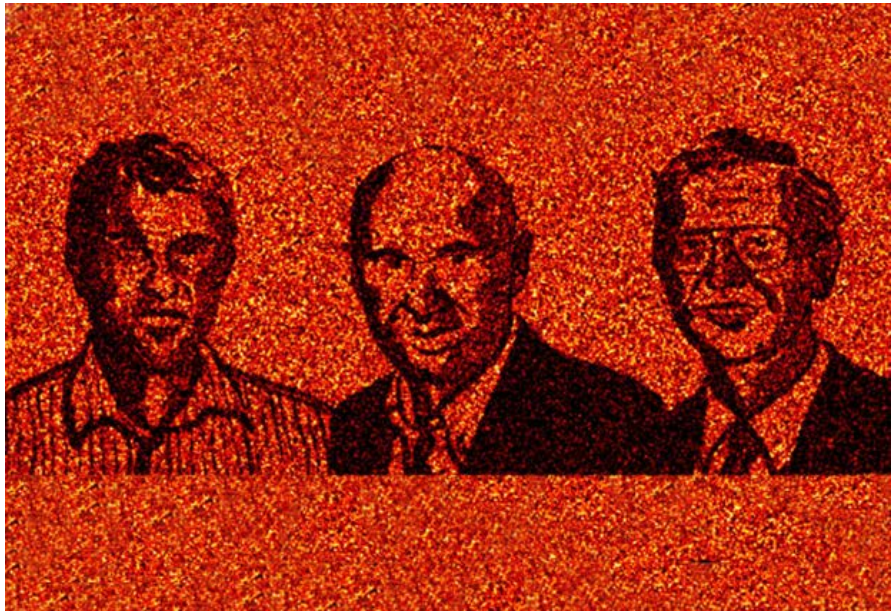


Highly sensitive (no background), suboptimal spectral properties.
Limited probes: luminol, dioxetanes (Shabat)

FLUORESCENT PROBES

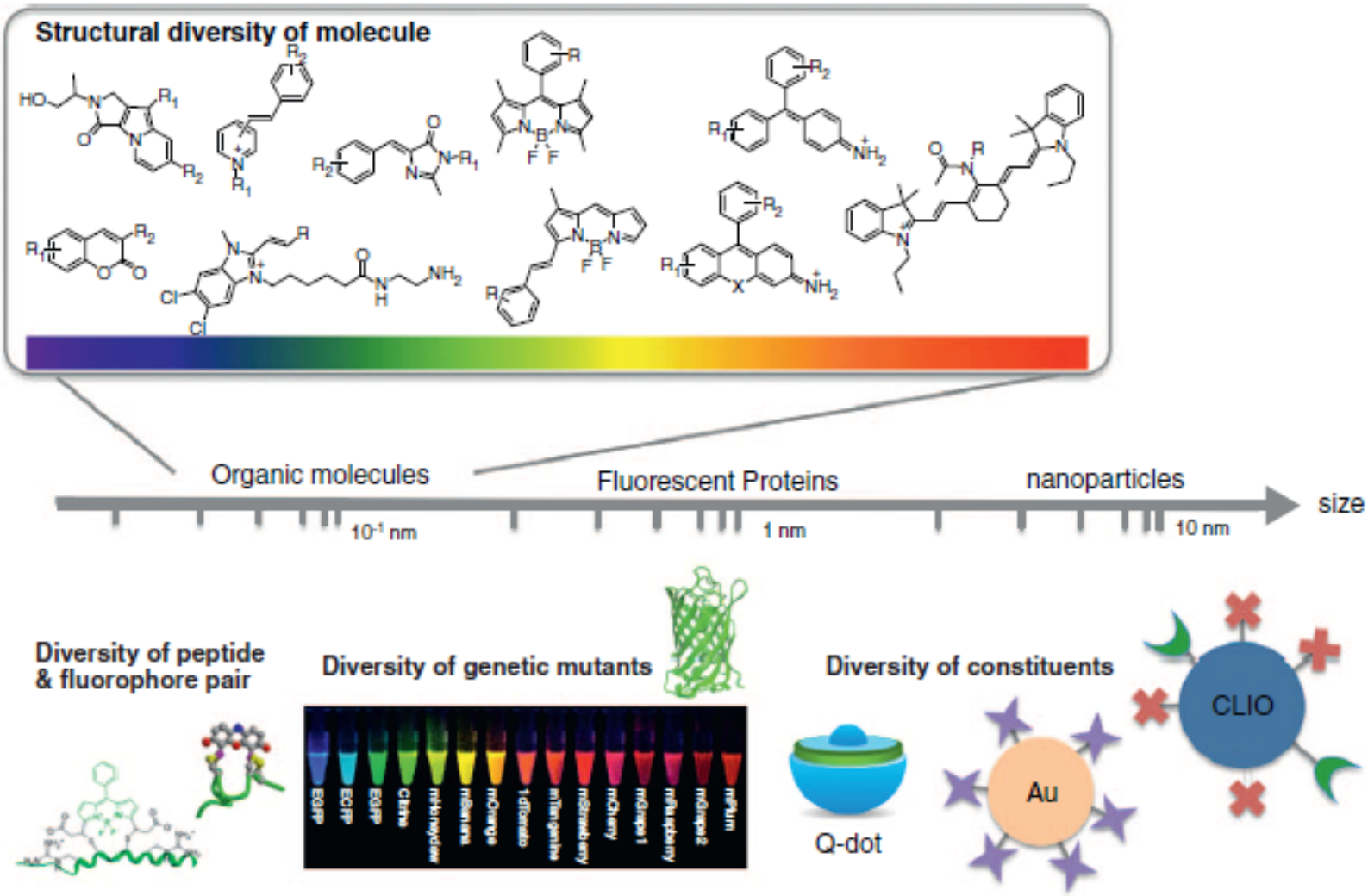


2008 Nobel Prize in Chemistry



2014 Nobel Prize in Chemistry

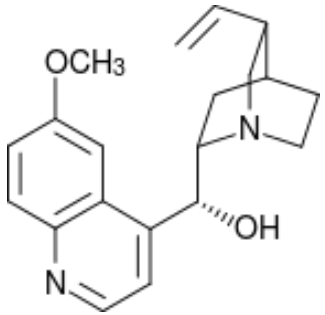
FLUORESCENT PROBES



Curr. Opin. Chem. Biol. 2011, 760.

FLUORESCENT PROBES

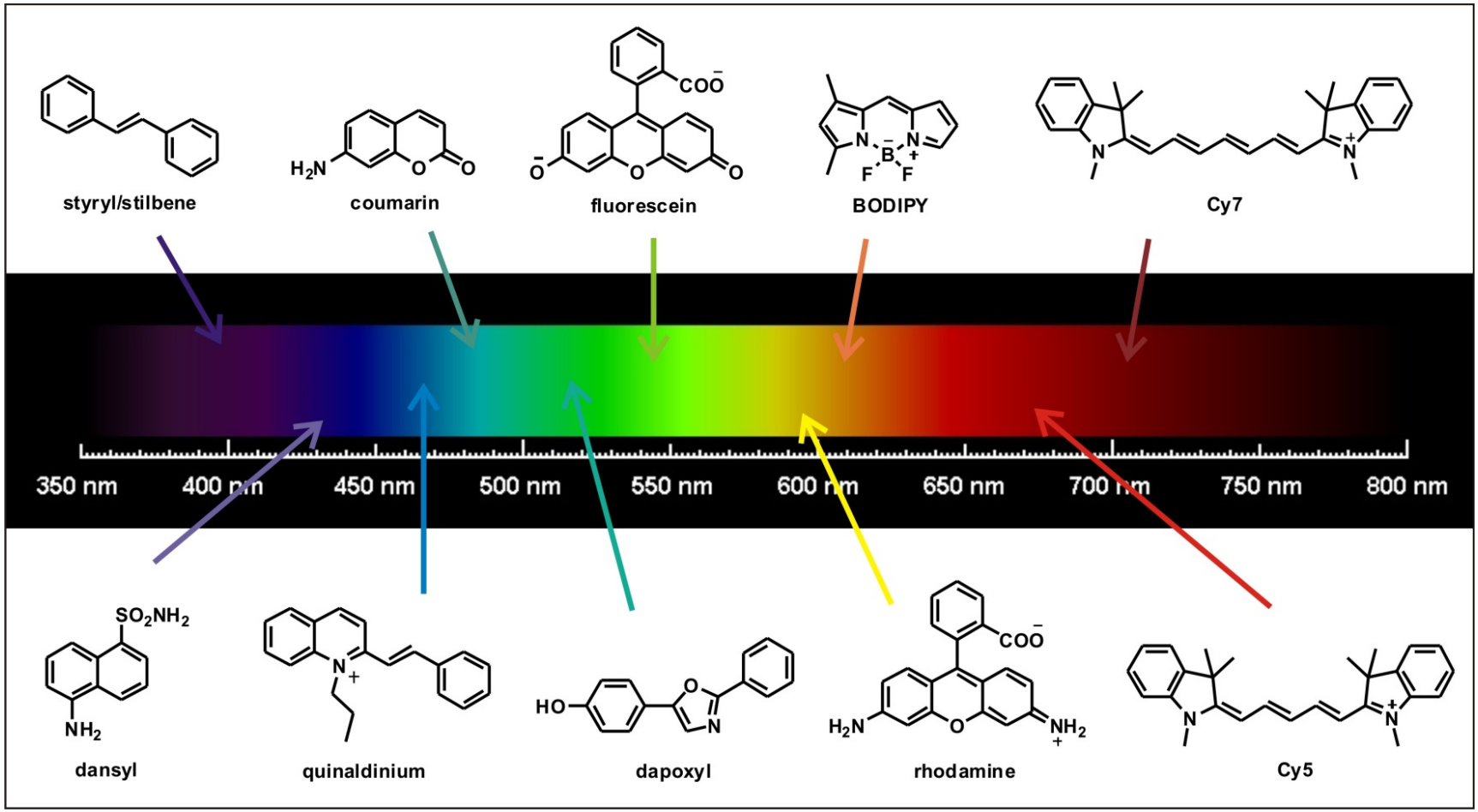
On a case of superficial colour presented by a homogeneous liquid internally colourless. By Sir John Frederick William Herschel, Philosophical Translation of the Royal Society of London (1845) 135:143–145. Received January 28, 1845 — Read February 13, 1845.



QUININE

light, or a white object, it yet exhibits in certain aspects, and under certain incidences of the light, an extremely vivid and beautiful celestial blue colour,

FLUORESCENT PROBES

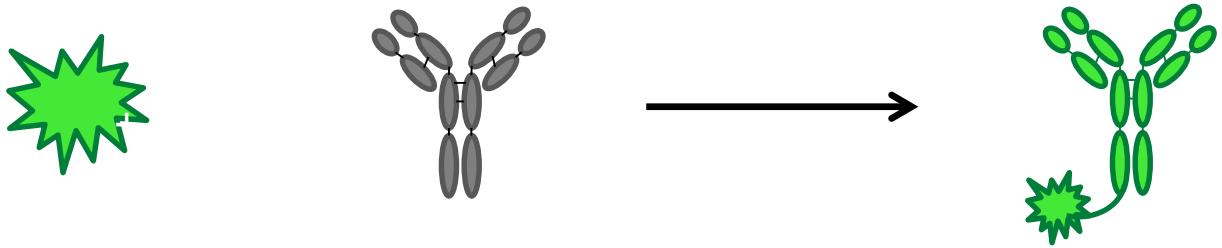


Chem. Rev. 2012, 4391.

FLUORESCENT PROBE DEVELOPMENT

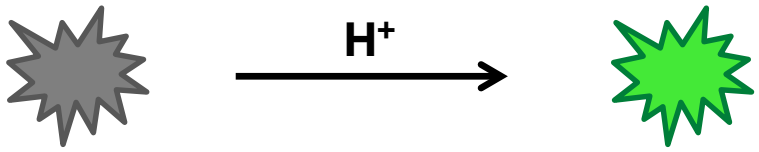
1. Labelling agents

   Always fluorescent

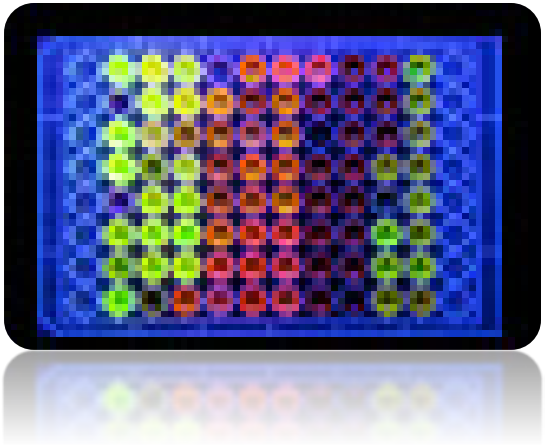


2. Activatable agents

   Activatable by pH, ROS, proteases….

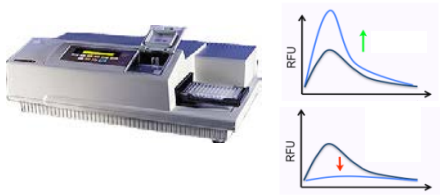


DIVERSITY-ORIENTED FLUORESCENT LIBRARIES

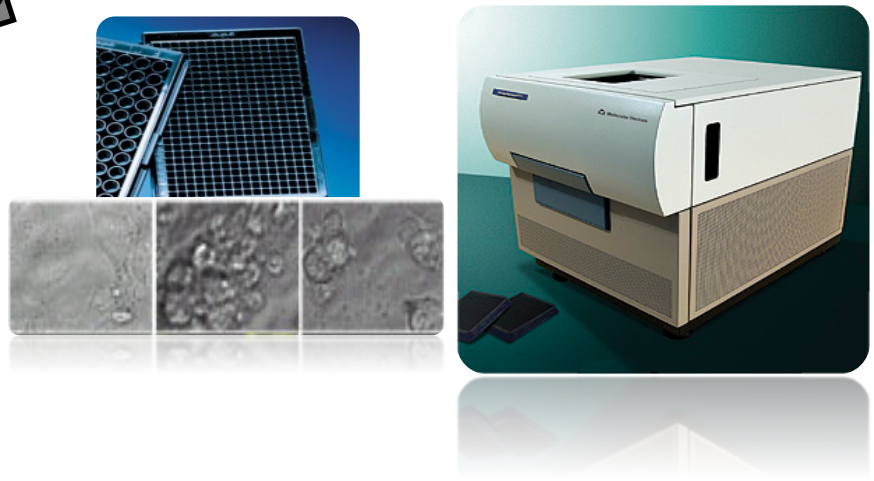


preparation of
fluorescent libraries

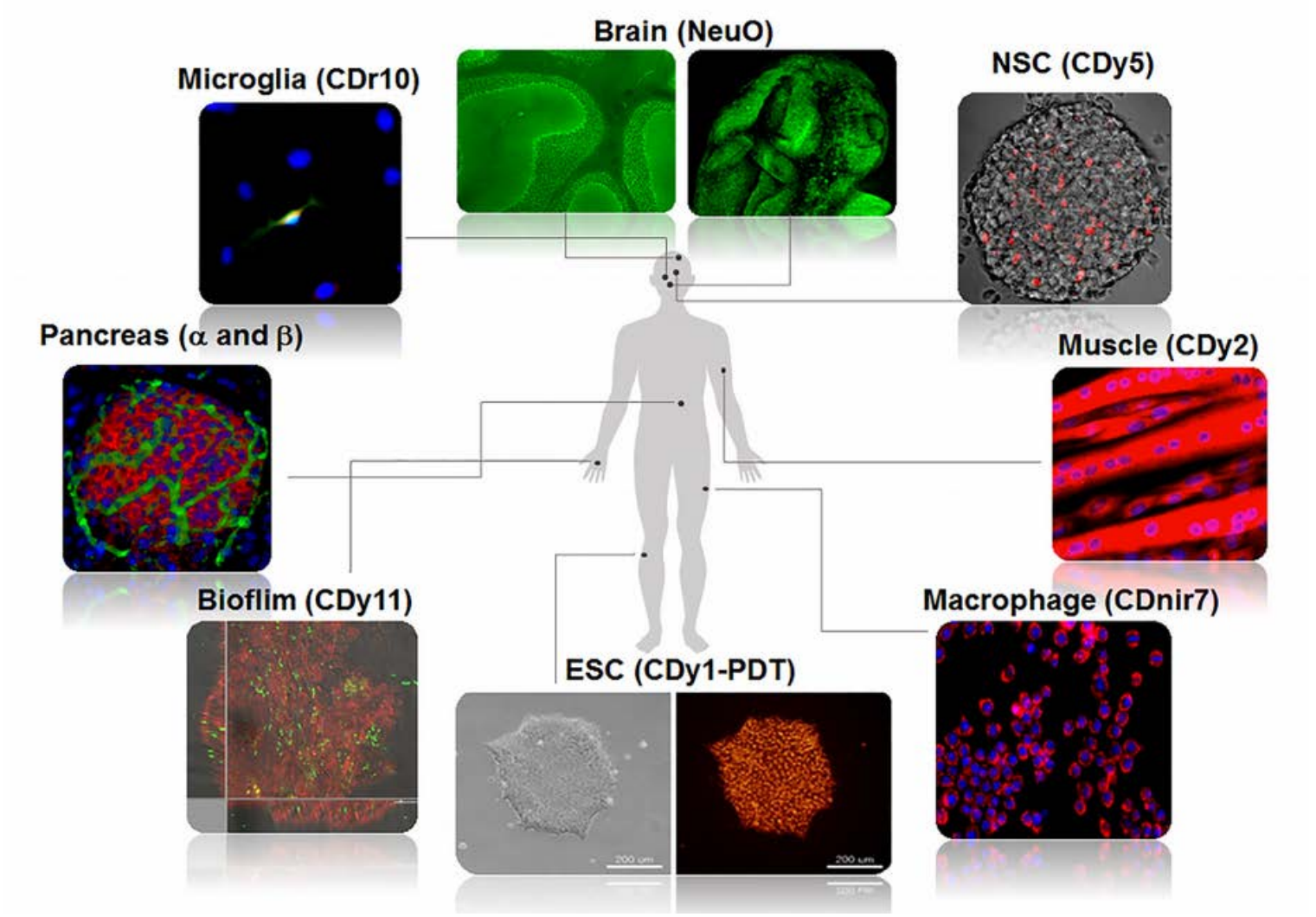
unbiased in vitro HT-screening



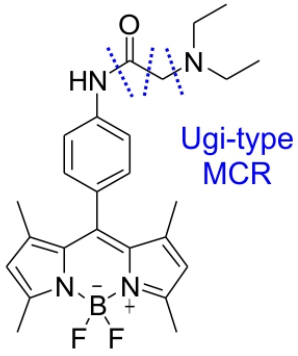
unbiased cell imaging HT-screening

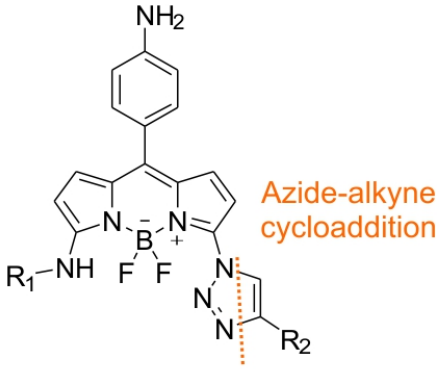


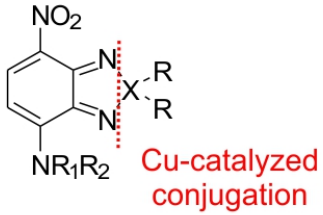
DIVERSITY-ORIENTED FLUORESCENT LIBRARIES

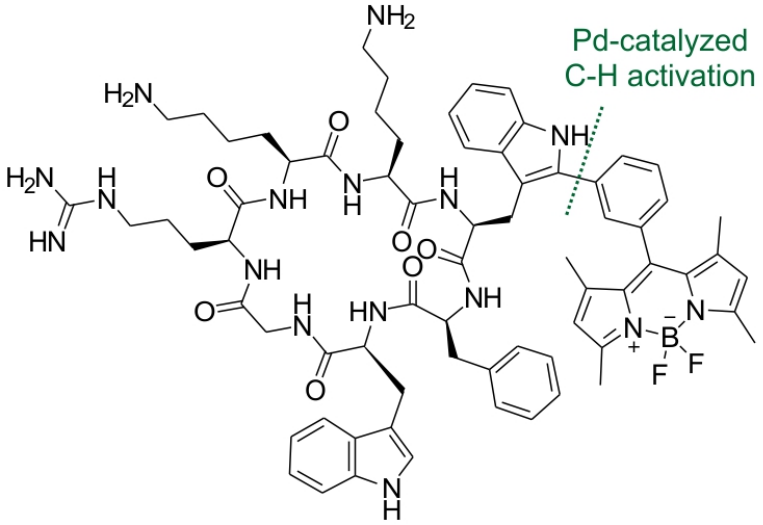


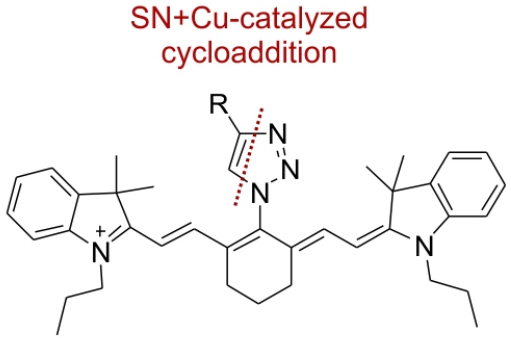
A TOOLBOX OF ACTIVATABLE FLUOROPHORES





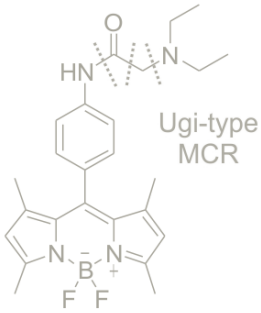


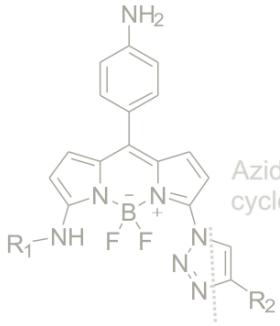


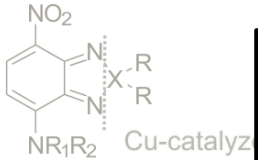


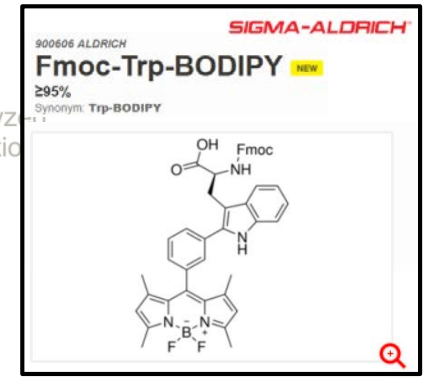
A TOOLBOX OF ACTIVATABLE FLUOROPHORES

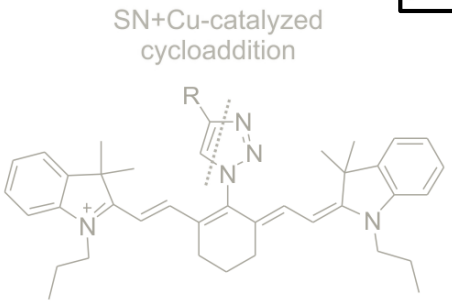
BioLegend®

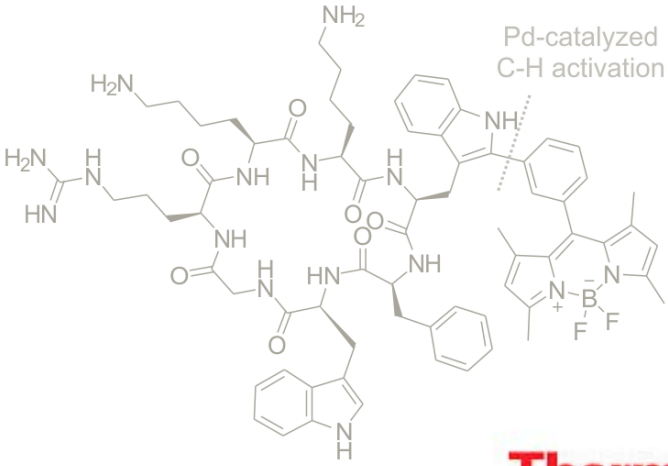












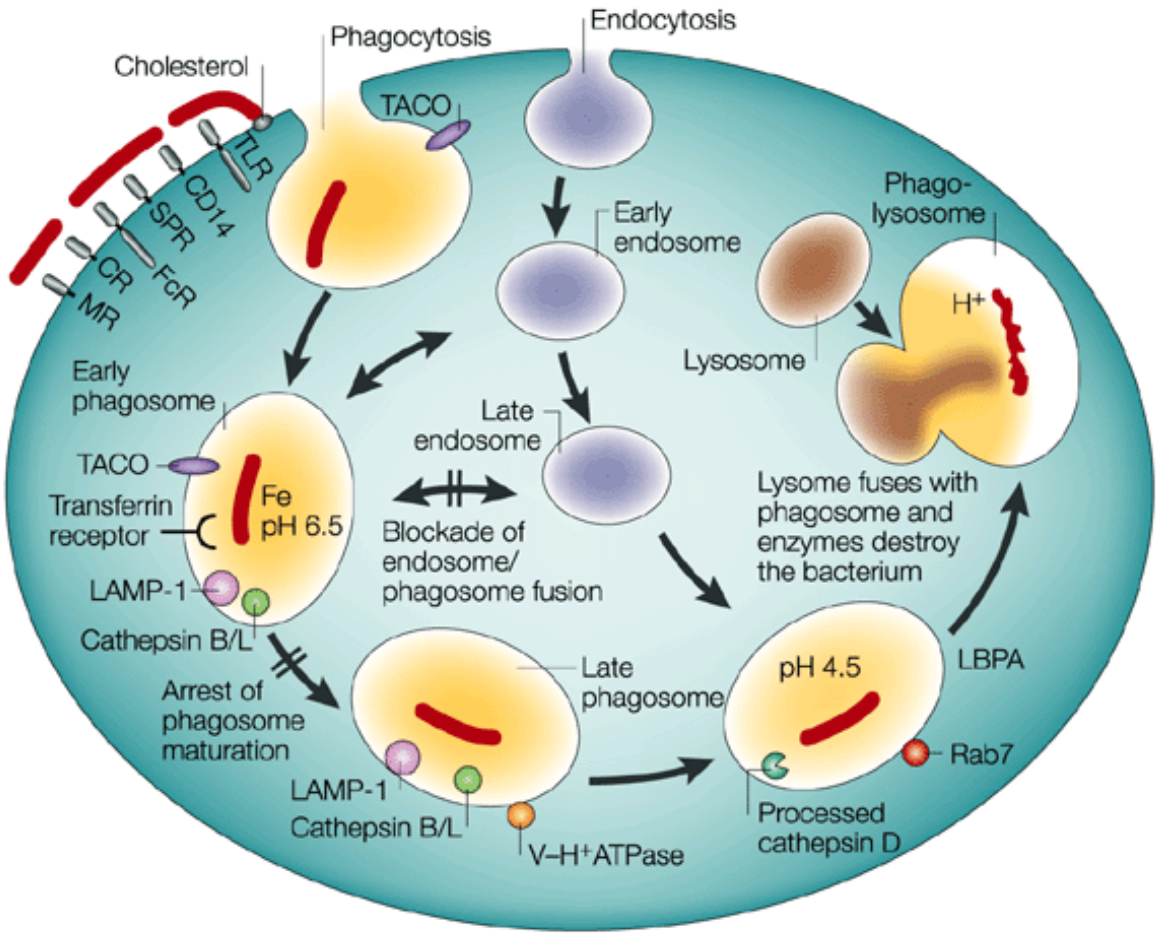
MERCK

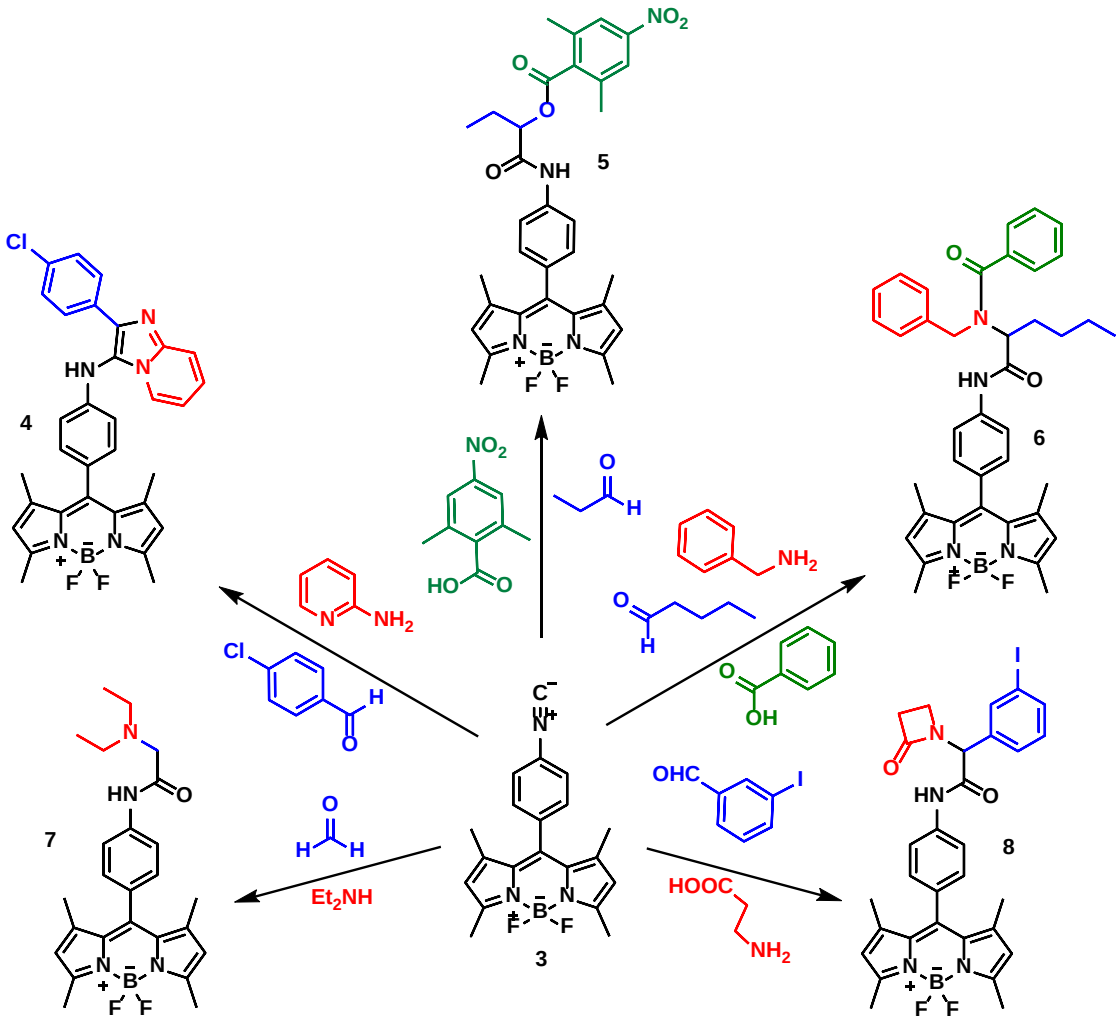
crb CAMBRIDGE RESEARCH BIOCHEMICALS

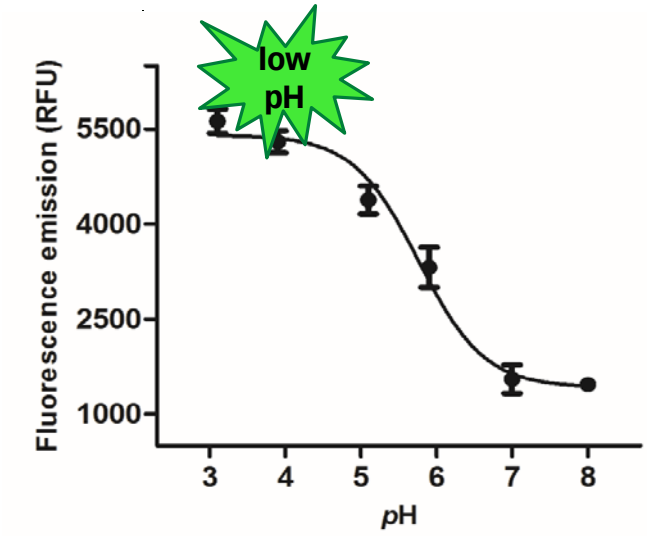
ThermoFisher SCIENTIFIC

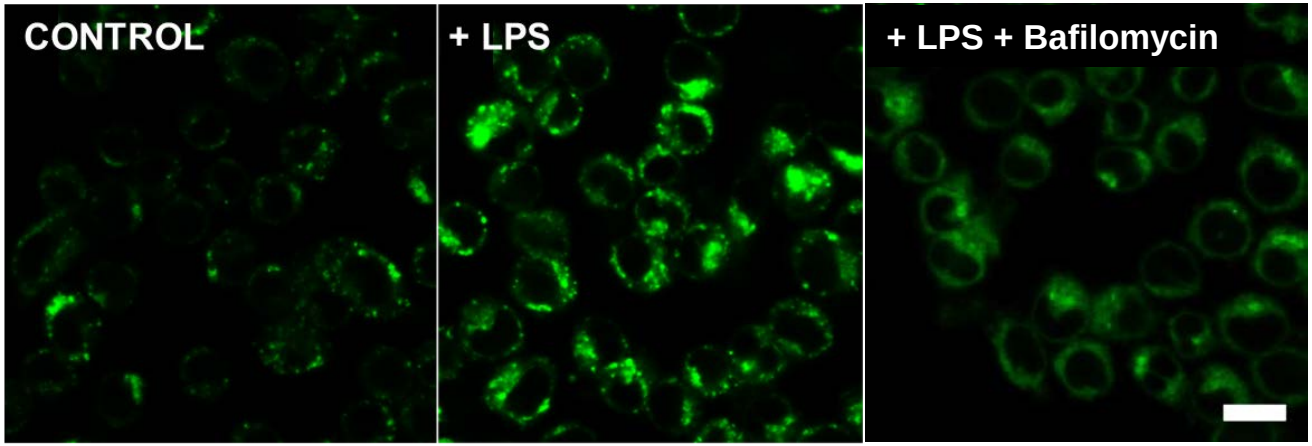
7 patents (PCT) since 2012

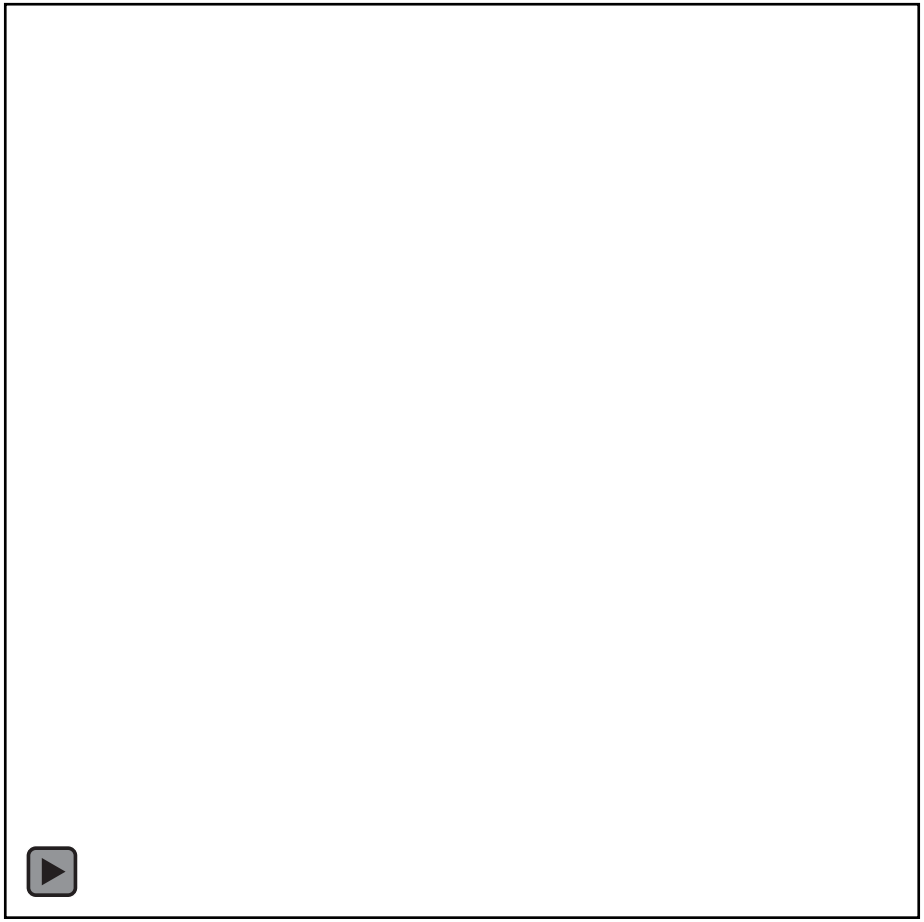
MACROPHAGES IN CANCER




NO2
O
O
NH
5
Cl
N
N
HN
4
O
N
HN
O
6
NO2
HO
O
O
H
NH2
N
NH2
Cl
H
O
O
H
O
OH
C
N
I
N
O
HN
O
7
OHC
I
O
H
H
Et2NH
HOOC
NH2
3
8
F
F
B

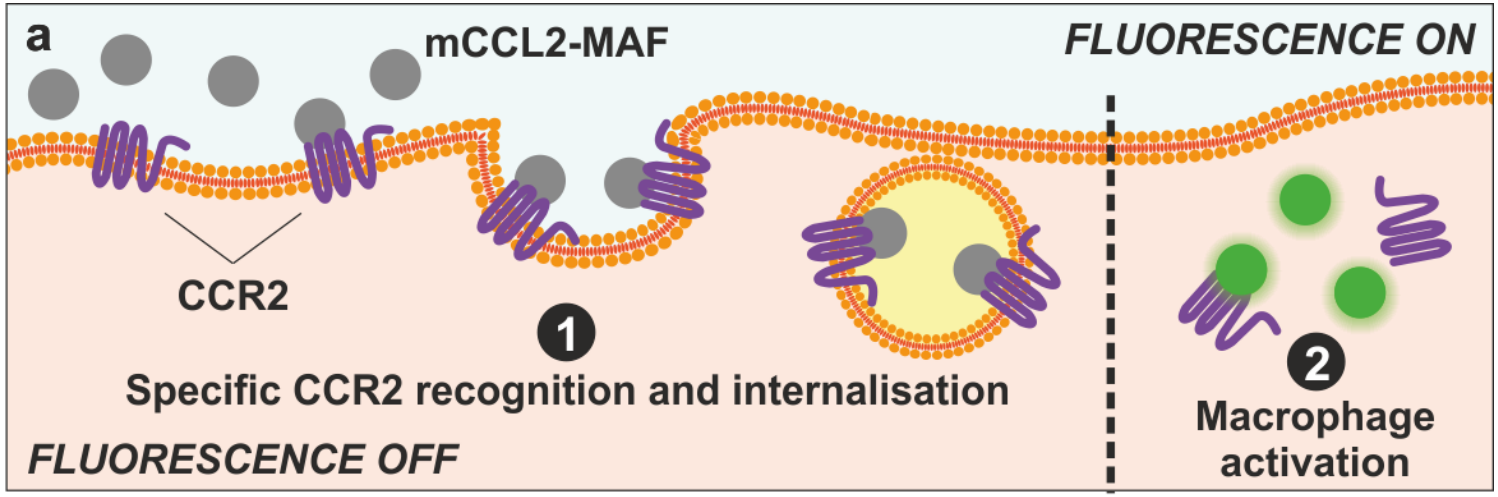

low
pH
Fluorescence emission (RFU)
5500
4000
2500
1000
3
4
5
6
7
8
pH


CONTROL
+ LPS
+ LPS + Bafilomycin

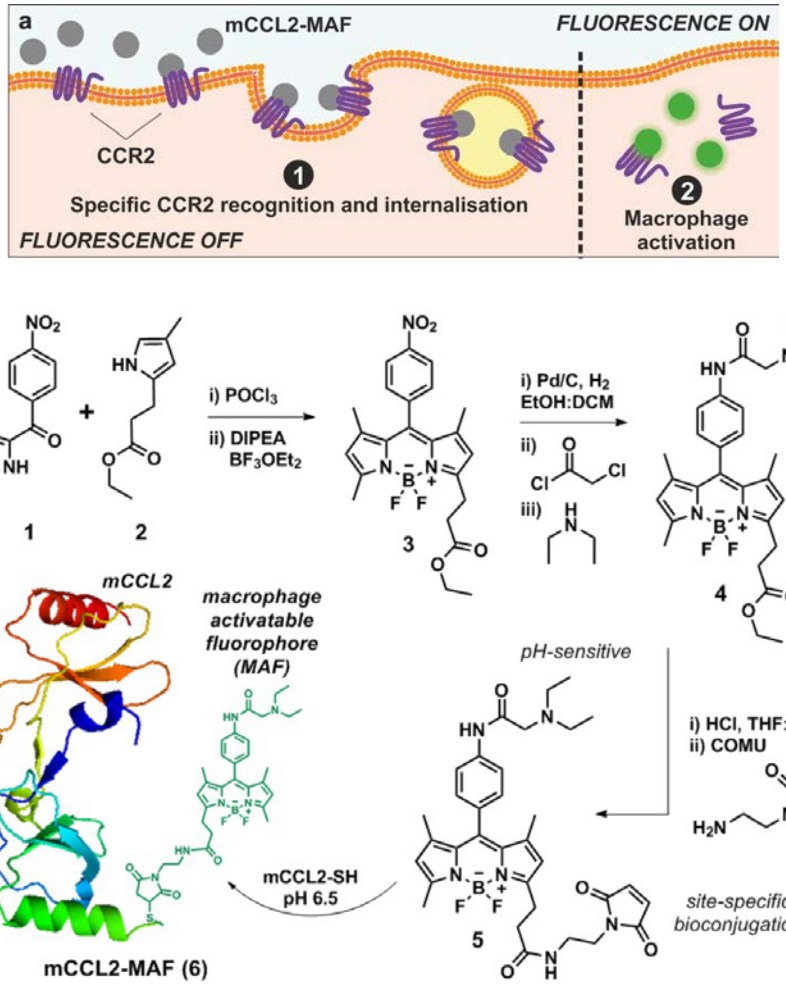


METASTASIS-ASSOCIATED MACROPHAGES

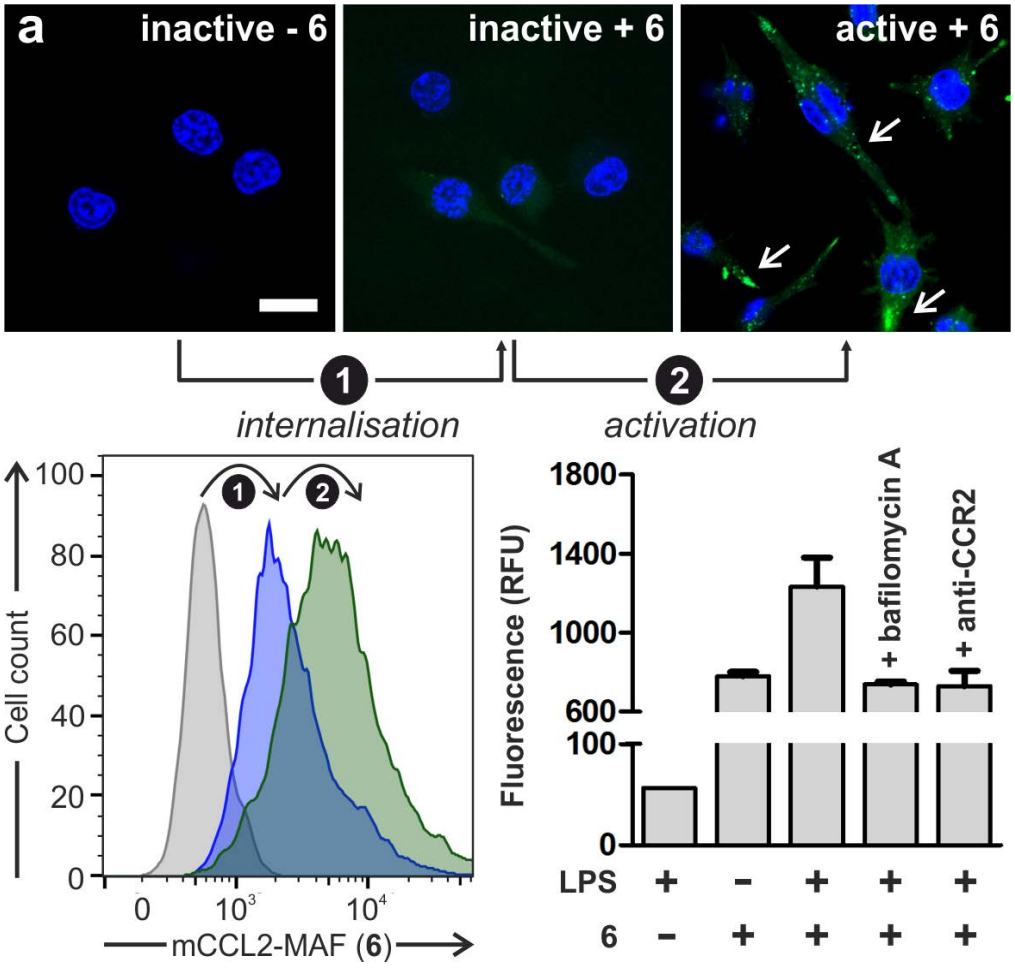
Logic gates to target disease-specific subpopulations of cells



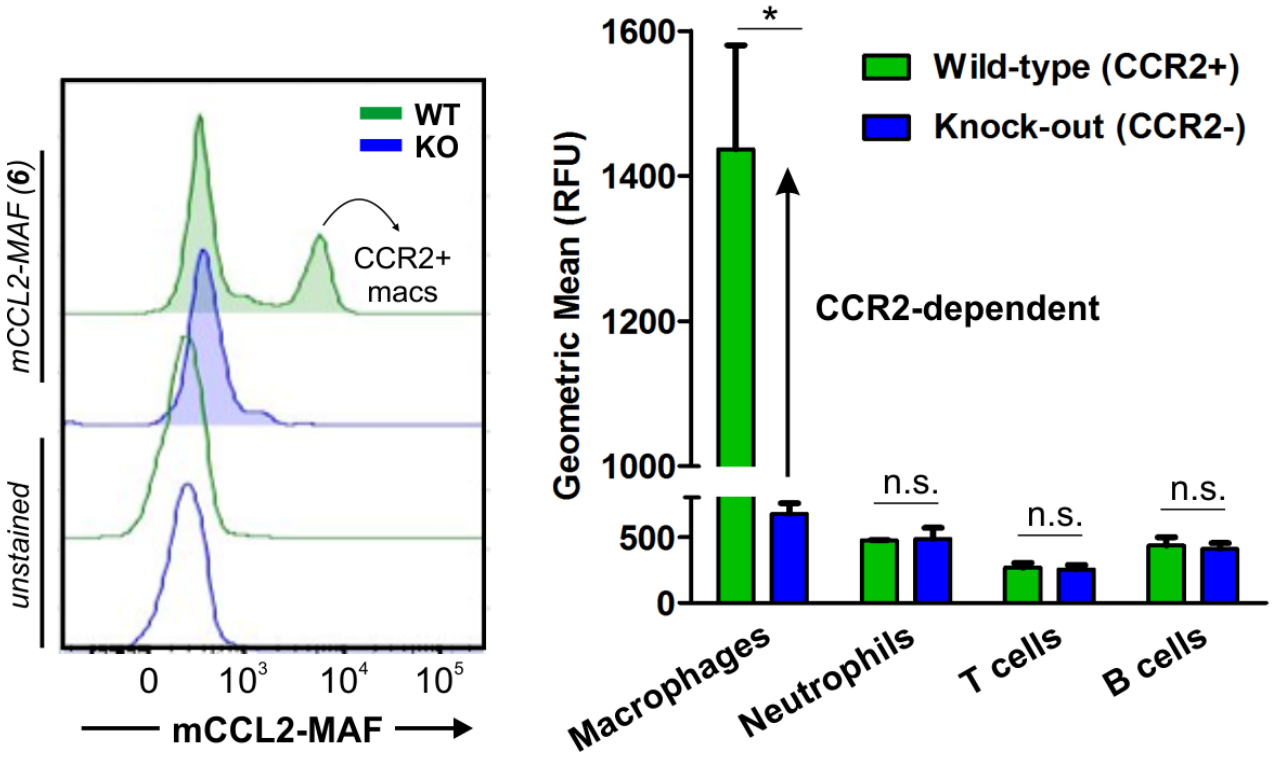
METASTASIS-ASSOCIATED MACROPHAGES



METASTASIS-ASSOCIATED MACROPHAGES

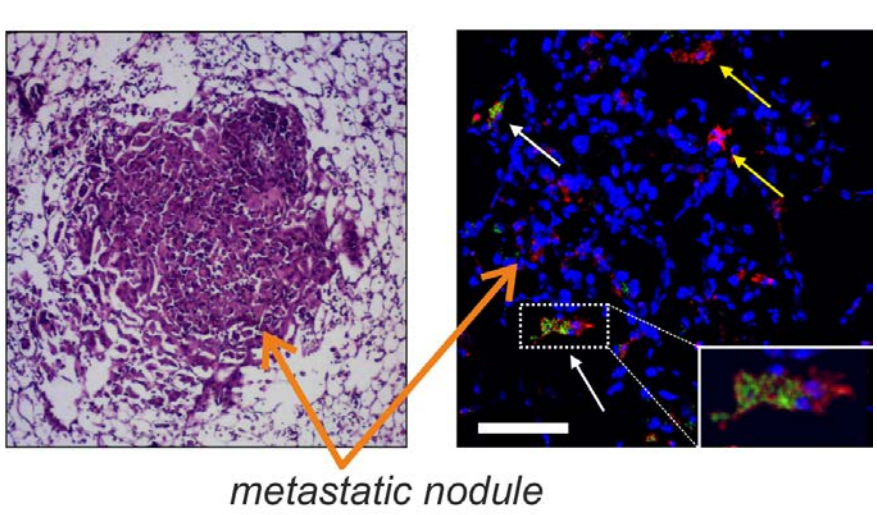


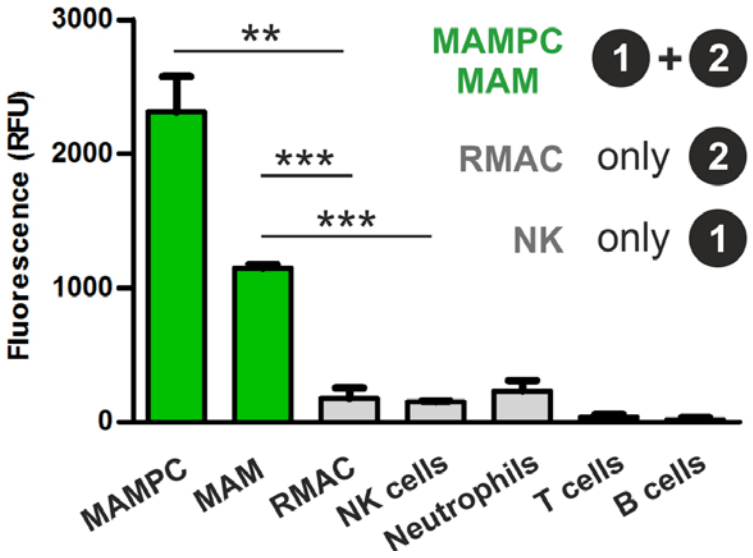
METASTASIS-ASSOCIATED MACROPHAGES



METASTASIS-ASSOCIATED MACROPHAGES

CCR2+ metastasis-associated macrophages in tumours



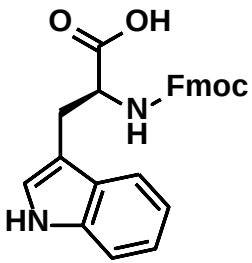


Angew. Chem. 2019, 16894.

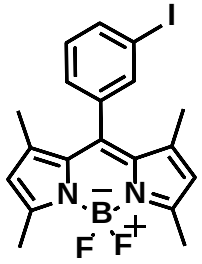
PEPTIDE-BASED FLUORESCENT PROBES

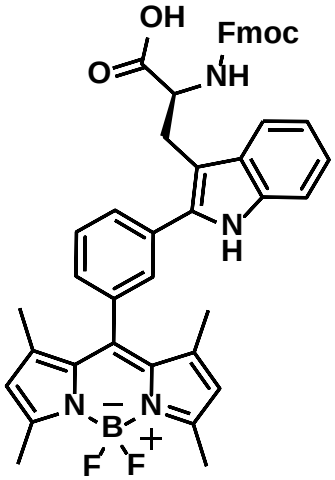
Can Zhao

ACS Comb. Sci. 2016, 689.



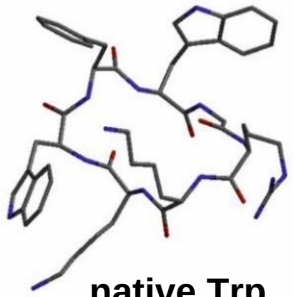
$Pd(OAc)_2$ (0.05 eq)
$AgBF_4$ (1.0 eq)
TFA (1.0 eq), DMF
M_W 80º C, 20'



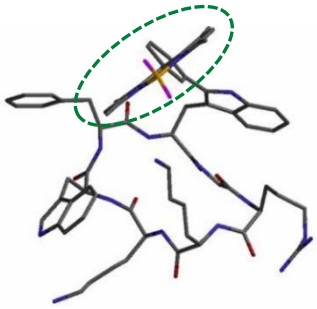


$\lambda_{abs./em.}$: 503/517 nm
QY (PBS): 0.03
QY (membranes): 0.22
ε: 121,000 $M^{-1}cm^{-1}$

SPPS

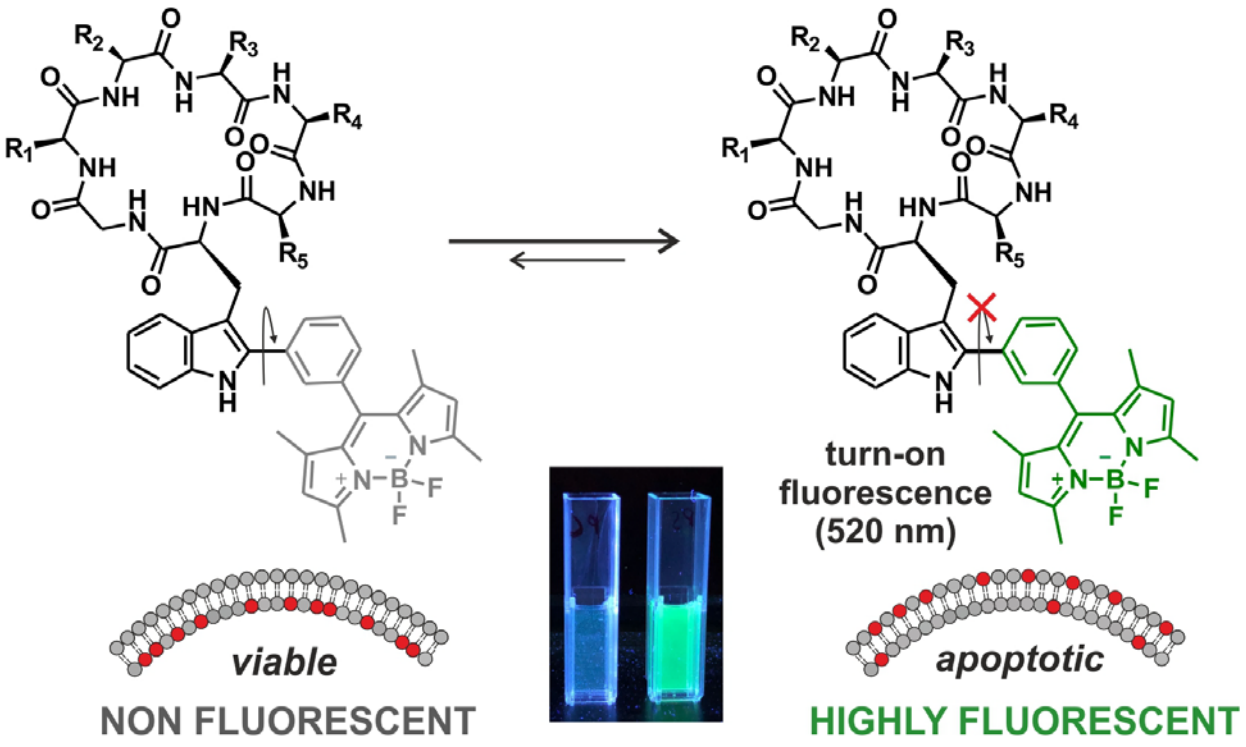


native Trp

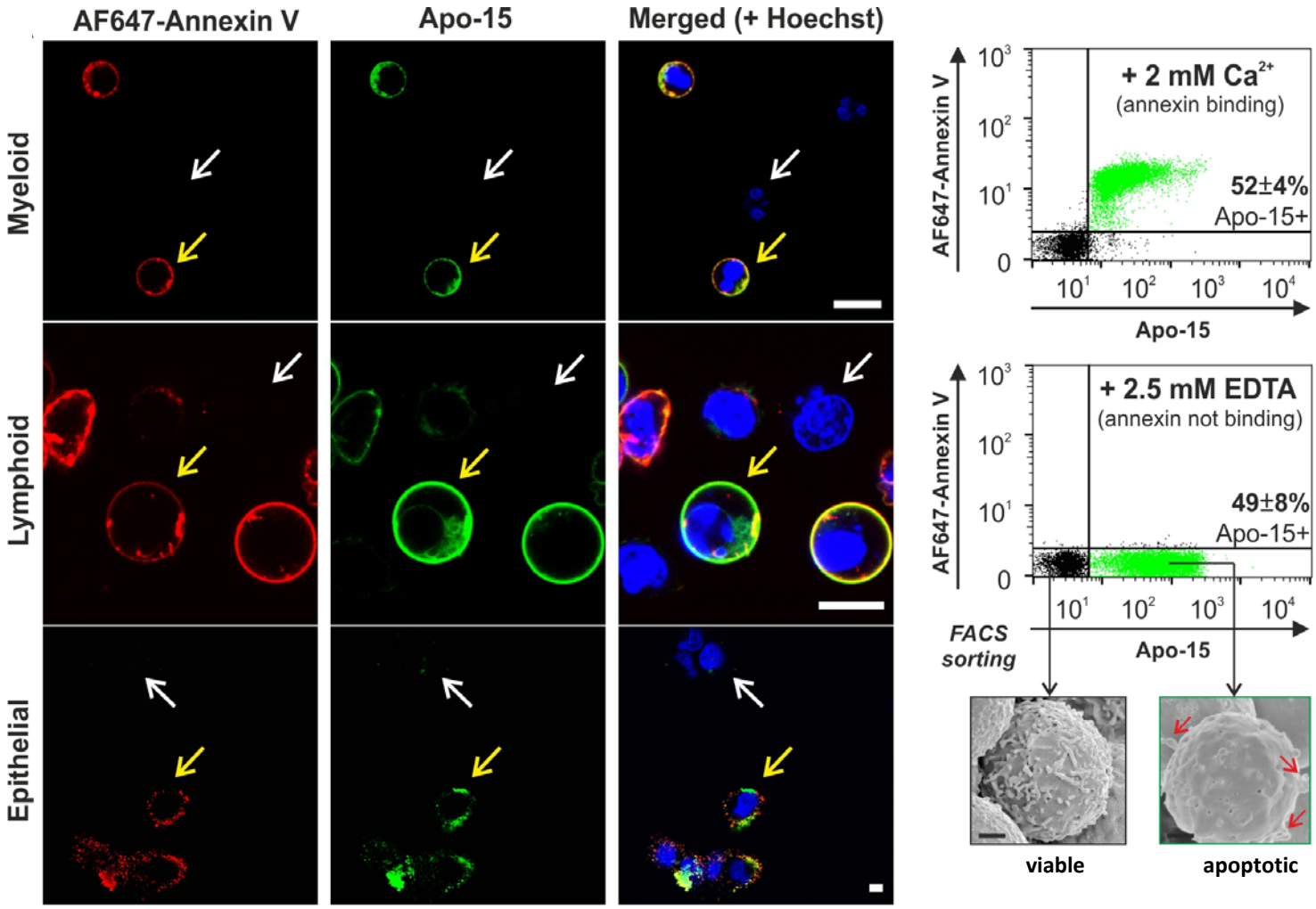


Trp-BODIPY

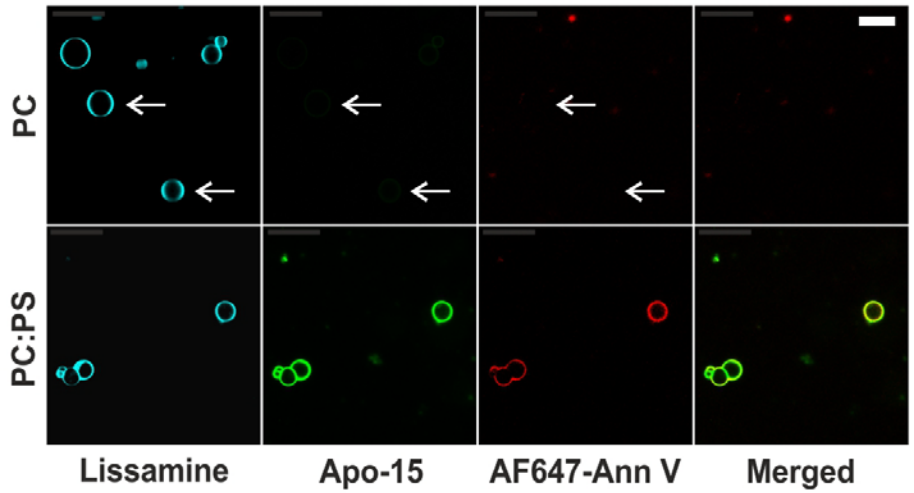
A PEPTIDE PLATFORM FOR IMAGING APOPTOSIS *IN VIVO*

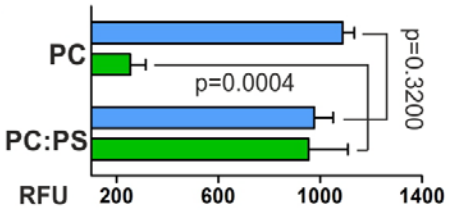


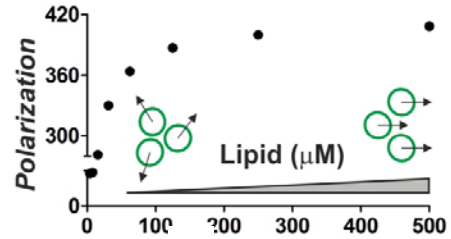
A PEPTIDE PLATFORM FOR IMAGING APOPTOSIS *IN VIVO*

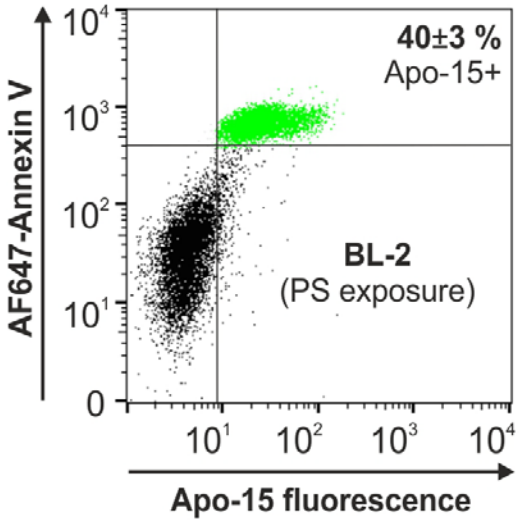


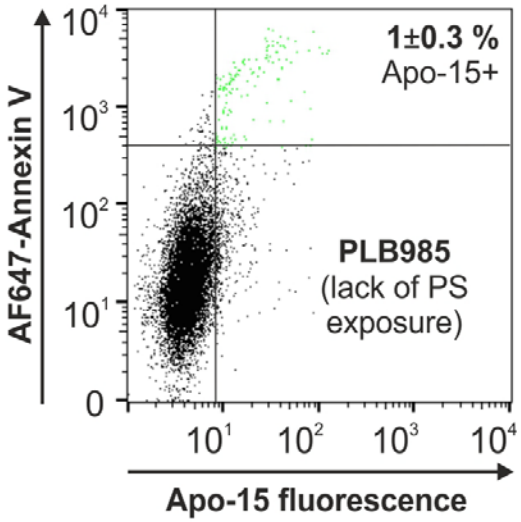
A PEPTIDE PLATFORM FOR IMAGING APOPTOSIS *IN VIVO*

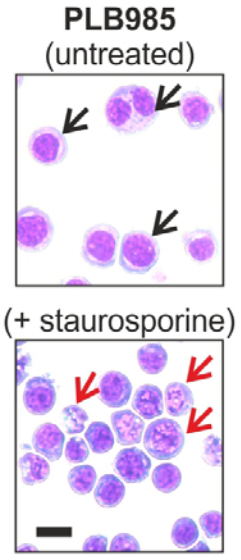




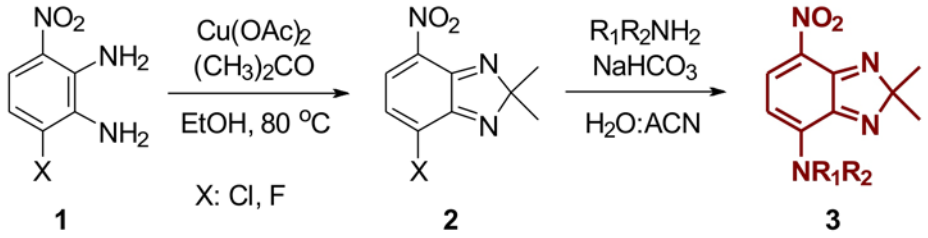






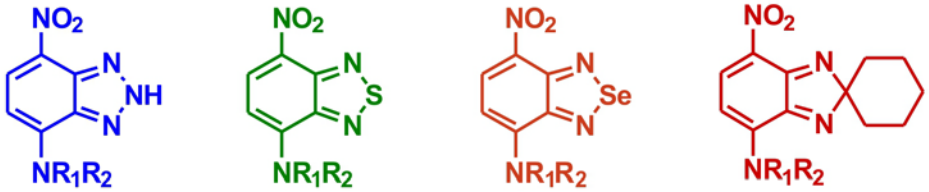


IMAGING METABOLITES: SCOTFLUORS

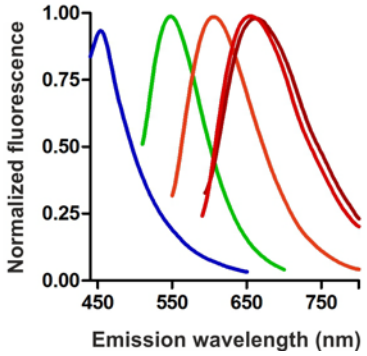


1 2 3

R_1: H or Et R_2: Pr or Et



4 5 6 7

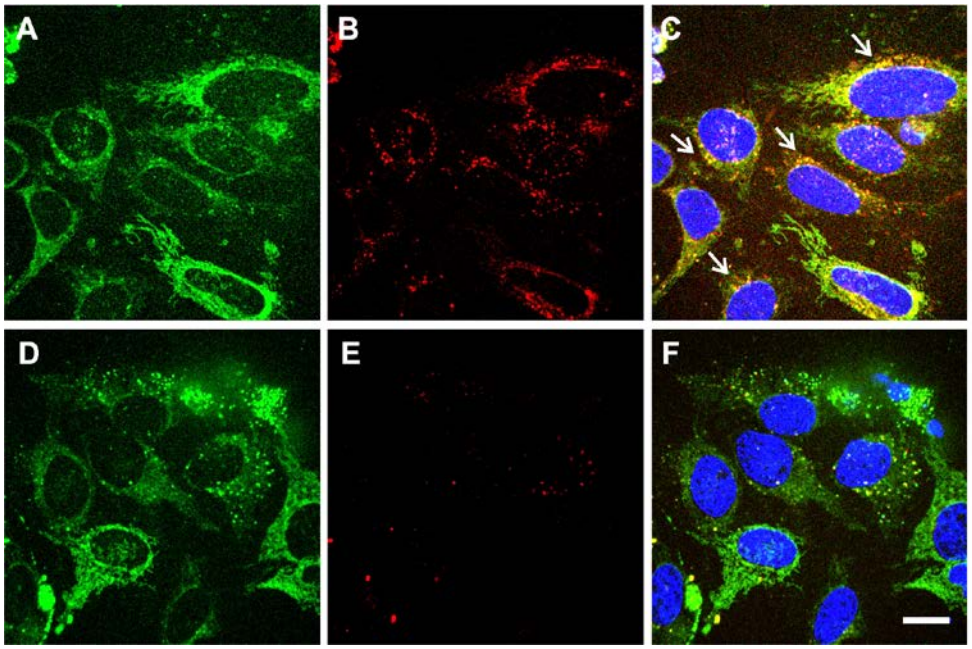


	$\lambda_{abs.}$ [a]	$\lambda_{em.}$ [a]	ε ($M^{-1}cm^{-1}$) [a]	Φ [b]
3	564	650	27,300	0.09
4	420	460	37,300	<0.01
5	476	548	14,600	0.54
6	510	606	16,300	0.20
7	546	650	28,300	0.11
NBD	486	542	15,400	0.55

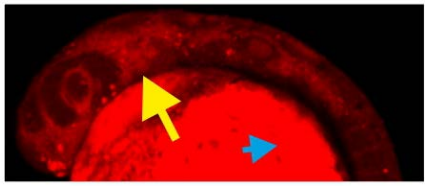
Angew. Chem. 2019.

IMAGING METABOLITES: GLUCOSE UPTAKE

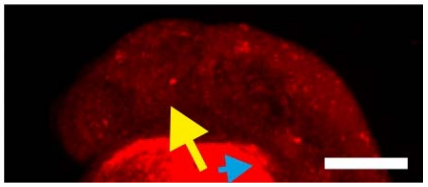
A red fluorescent glucose analog with functional retention in cells and *in vivo*



wildtype zebrafish



glut2 morpholino zebrafish

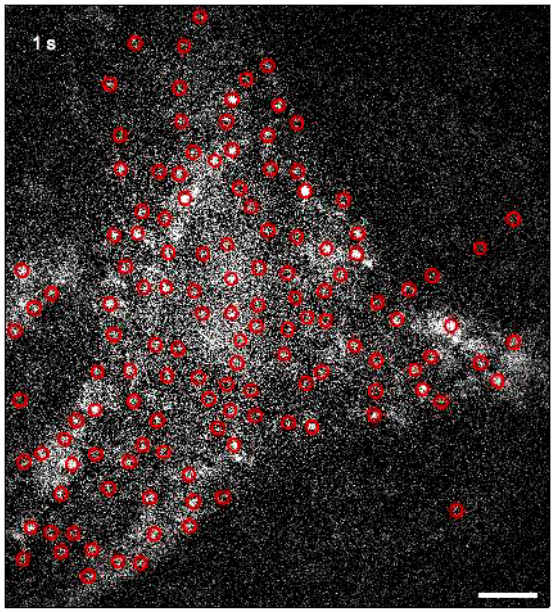


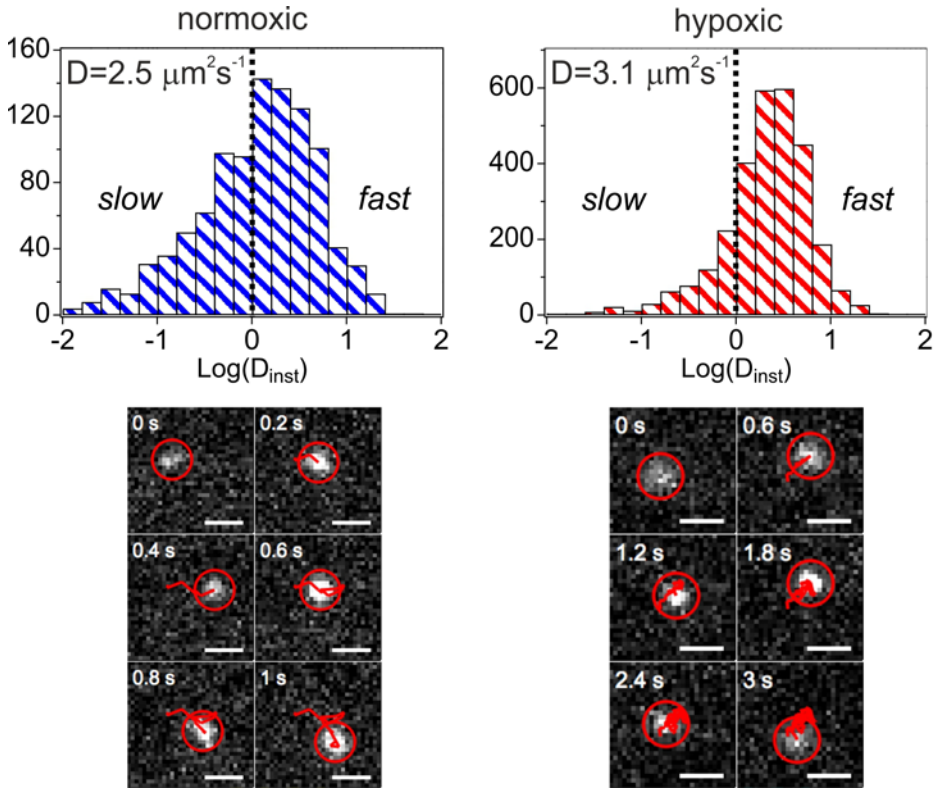
Collaboration: Kazuya Kikuchi (JP)

Angew. Chem. 2019.

IMAGING METABOLITES: LACTATE TRANSPORT

A fluorescent probe for imaging lactate in real time



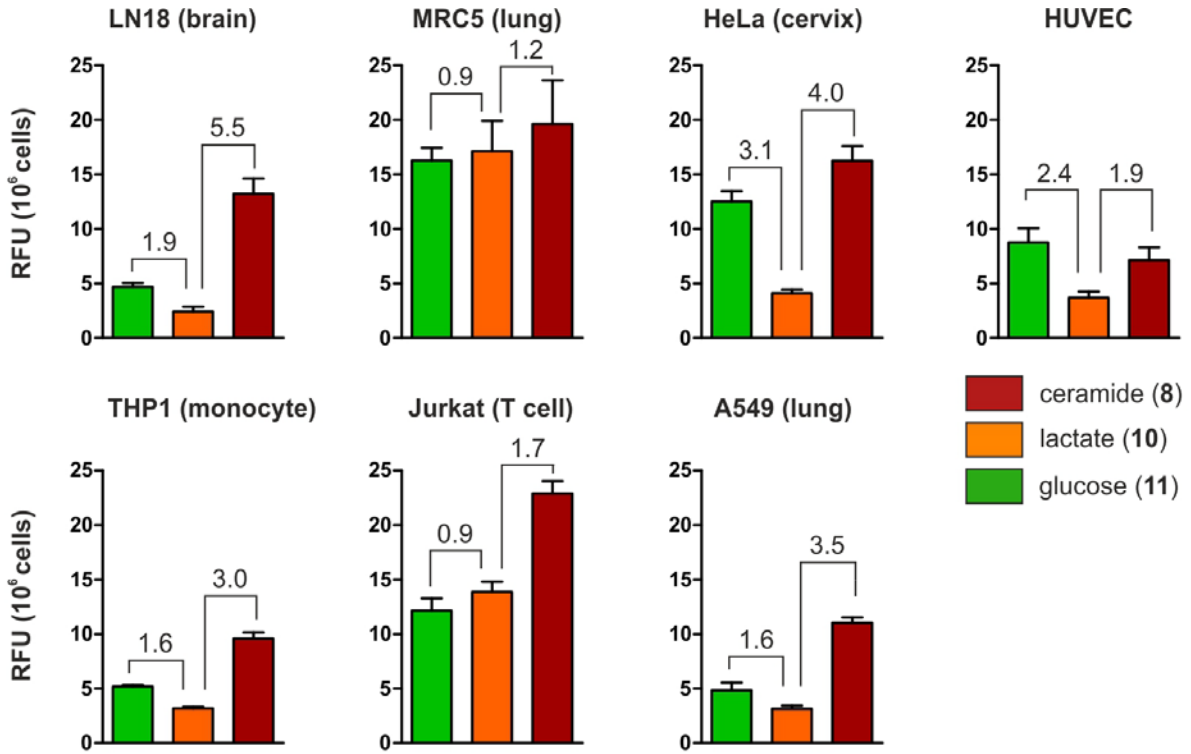


Collaboration: Mathew Horrocks (UK)

Angew. Chem. 2019.

IMAGING METABOLITES: CELL PROFILING

Multi-colour metabolic fingerprints of human cancer cell lines



Angew. Chem. 2019.