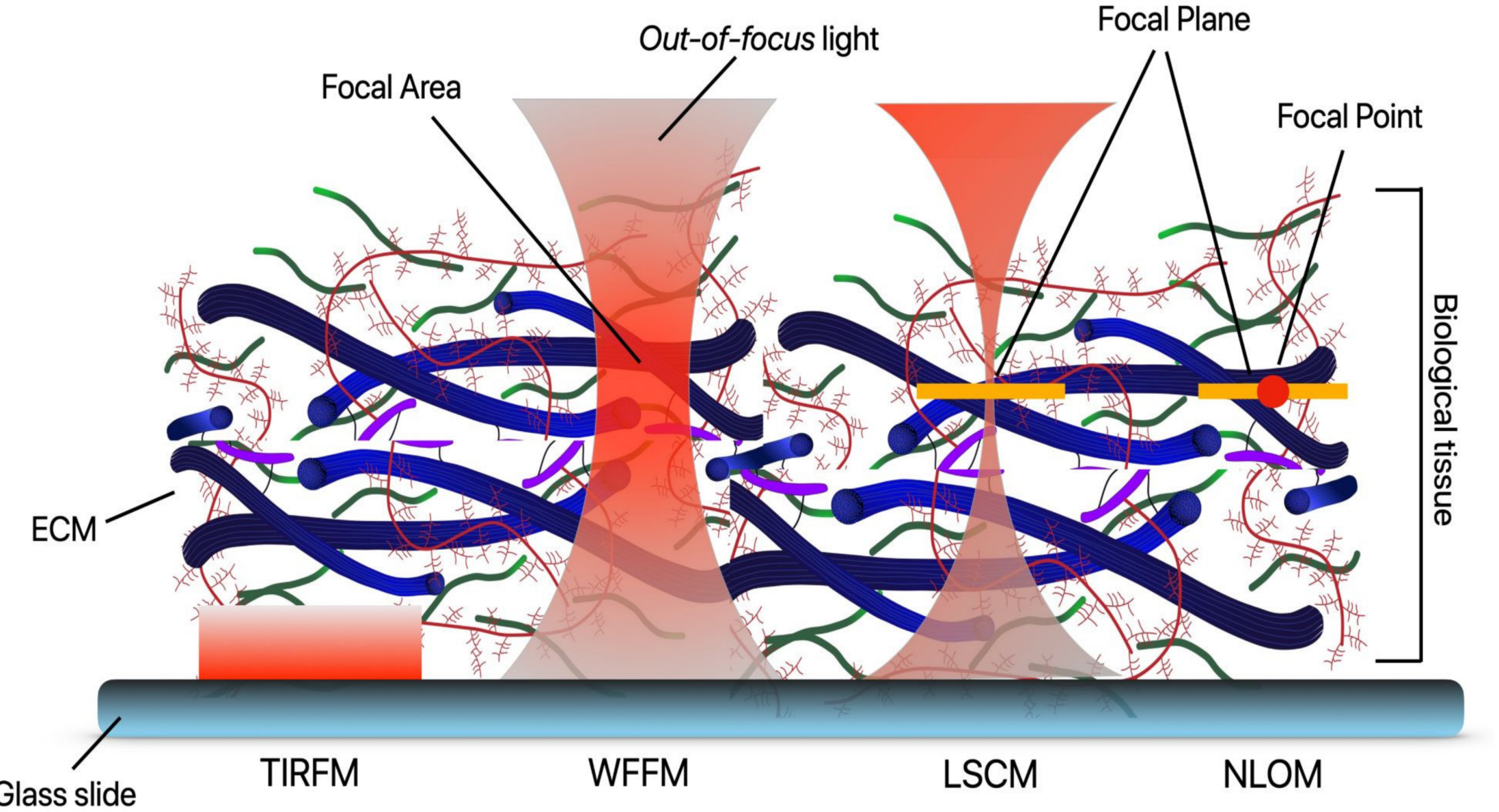
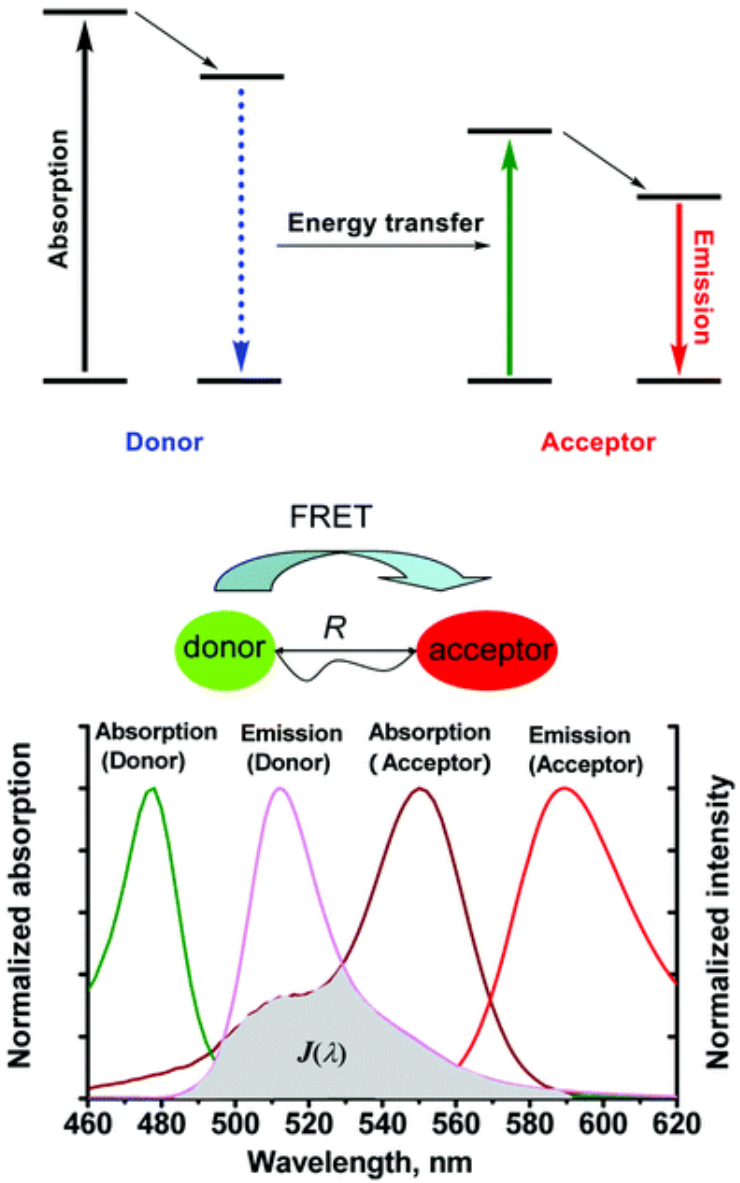


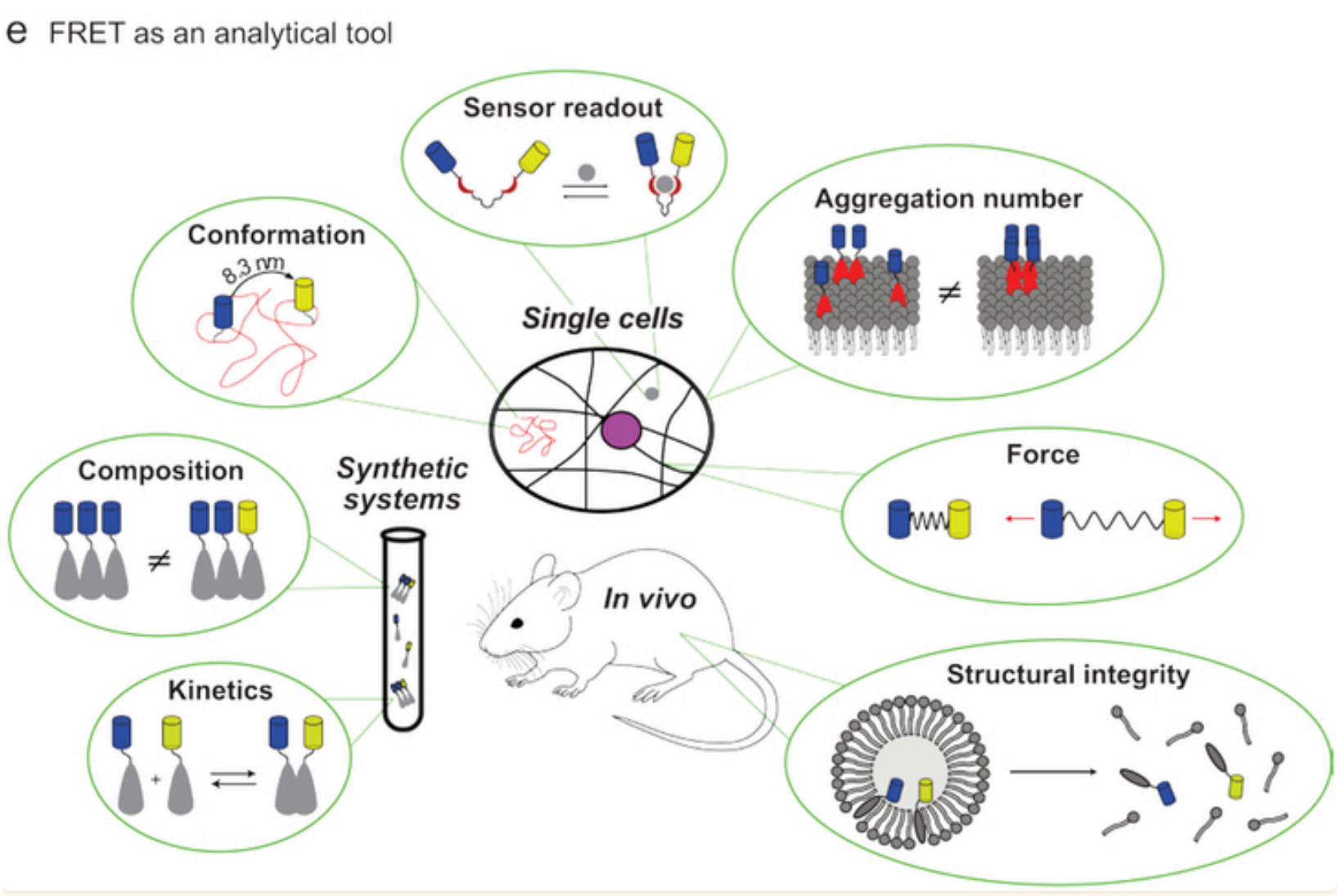
Cells 2021, *10*(7), 1760; https://doi.org/10.3390/cells10071760

“Förster Resonance energy transfer”

Denomina-se de **Transferência de energia por ressonância de Förster (FRET)**, ou **transferência de energia de ressonância por fluorescência (FRET)**, ou **transferência de energia por ressonância (RET)** o mecanismo de transferência de energia de forma não-radiativa entre dois cromóforos, sem a necessidade de reabsorção de radiação eletromagnética. De maneira geral um cromóforo doador, inicialmente no seu estado eletrônico excitado, pode transferir energia para um aceitador, outro cromóforo, através do acoplamento dipolo-dipolo não radiativo. A eficiência desta transferência de energia é inversamente proporcional à sexta potência da distância entre o doador e o aceitador por esse mecanismo, tornando extremamente sensível a pequenas distâncias, da ordem de 1 a 10 nm.



Scheme 1 The mechanism of Förster resonance energy transfer (FRET). *R* is the distance between the energy donor and acceptor, $J(\lambda)$ represents the degree of spectral overlap between the donor emission and the acceptor absorption. Reproduced with permission from ref. 3 (*Acc. Chem. Res.*, 2013, **46**, 1462–1473). Copyright (2013) American Chemical Society.



Chem Soc Rev. 2018 Sep 17; 47(18): 7027–7044. doi: 10.1039/c8cs00278a

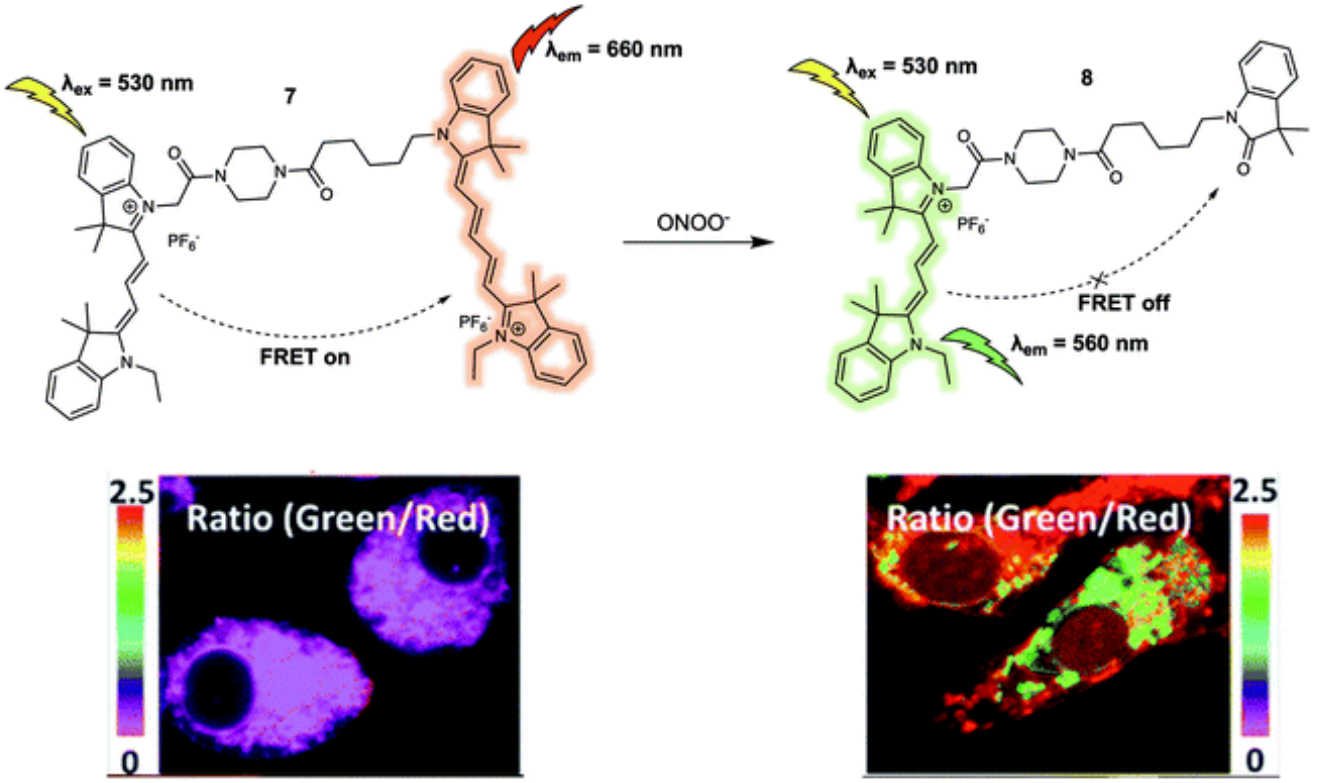


Fig. 6 Cy3/Cy5 (energy donor/acceptor)-based FRET fluorescent probe **7** developed for the ratiometric detection of $ONOO^-$. Also shown is the structure of its daughter product, **8**. Reproduced with permission from ref. 12 (*J. Am. Chem. Soc.*, 2016, **138**, 10778–10781). Copyright (2016) American Chemical Society.

Chem Soc Rev (2020) https://doi.org/10.1039/C9CS00318E

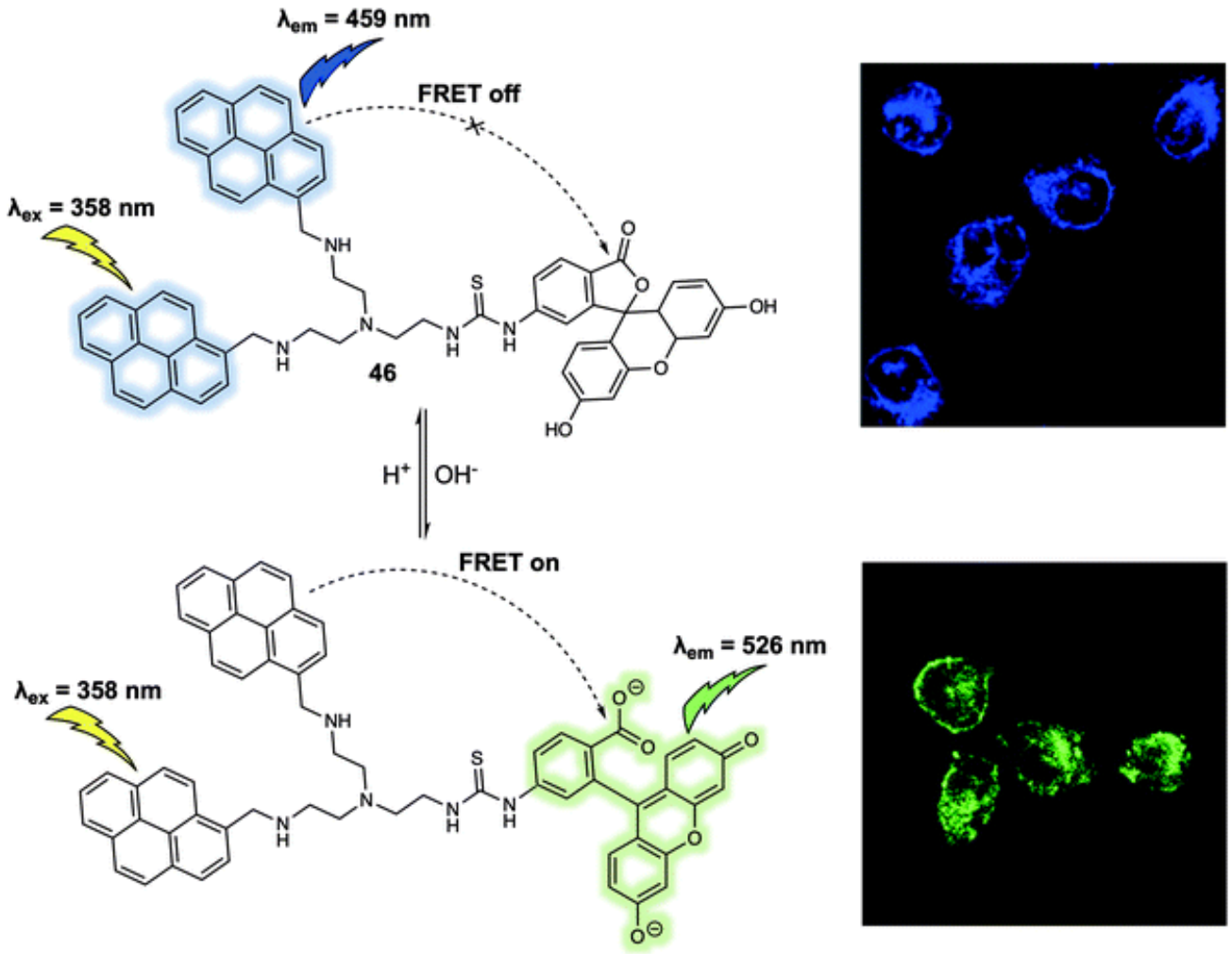


Fig. 28 FRET-based probe **46** that permit monitoring of pH changes in HeLa cells. Reproduced with permission from ref. 38 (*Anal. Chem.*, 2014, **86**, 10389–10396). Copyright (2014) American Chemical Society.

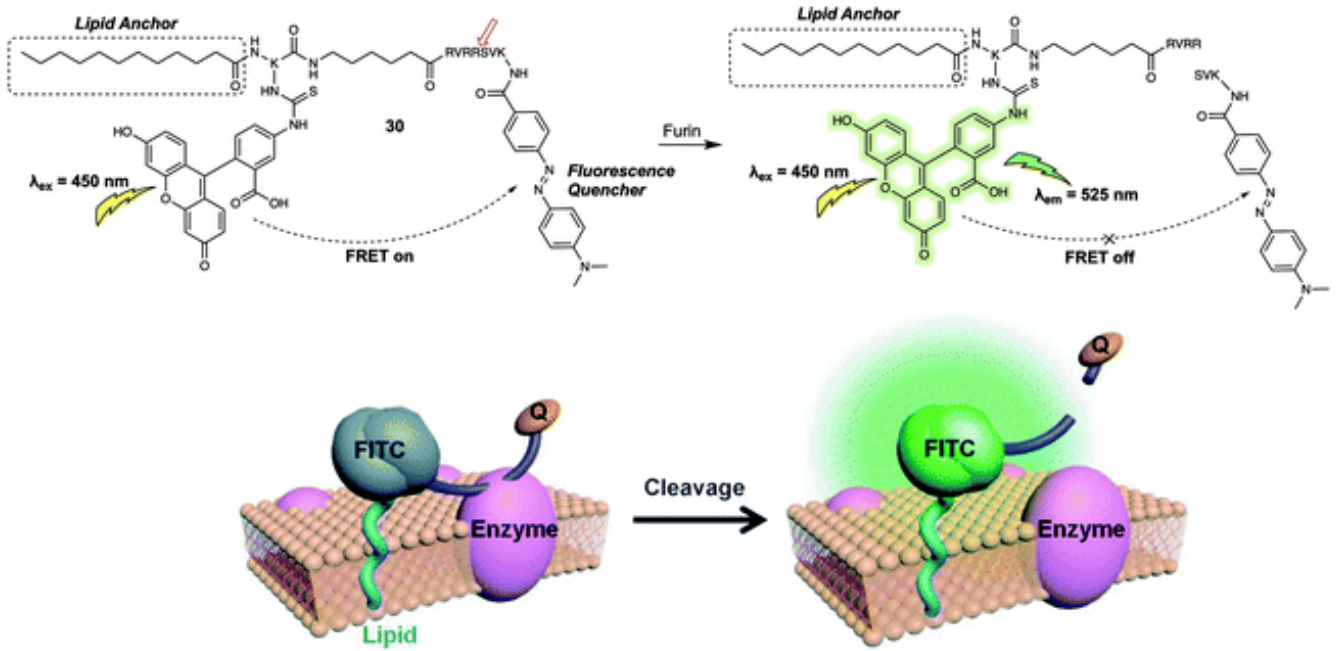


Fig. 19 Probe **30** for the detection of cell-surface-associated furin. Reproduced with permission from ref. 29 (*Angew. Chem., Int. Ed.*, 2014, **53**, 14357–14362). Copyright (2014) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

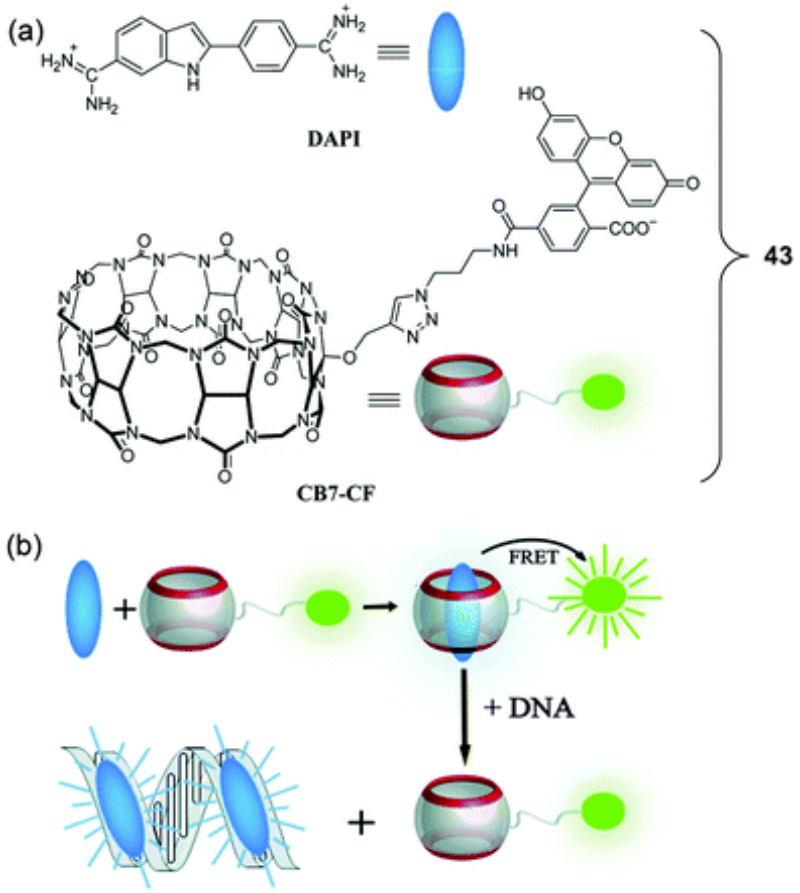


Fig. 26 (a) Molecular structures of **DAPI** and **CB7-CF**. (b) Schematic illustration of the resulting DNA chemosensing ensemble, which relies on a FRET process between DAPI (donor) and CB7-carboxyfluorescein (acceptor), as well as the relative affinities of the DAPI guest for the CB7 host and double-stranded DNA (dsDNA). Reproduced with permission from ref. 36 (*Chem. Commun.*, 2019, **55**, 671–674). Copyright (2019) The Royal Society of Chemistry.

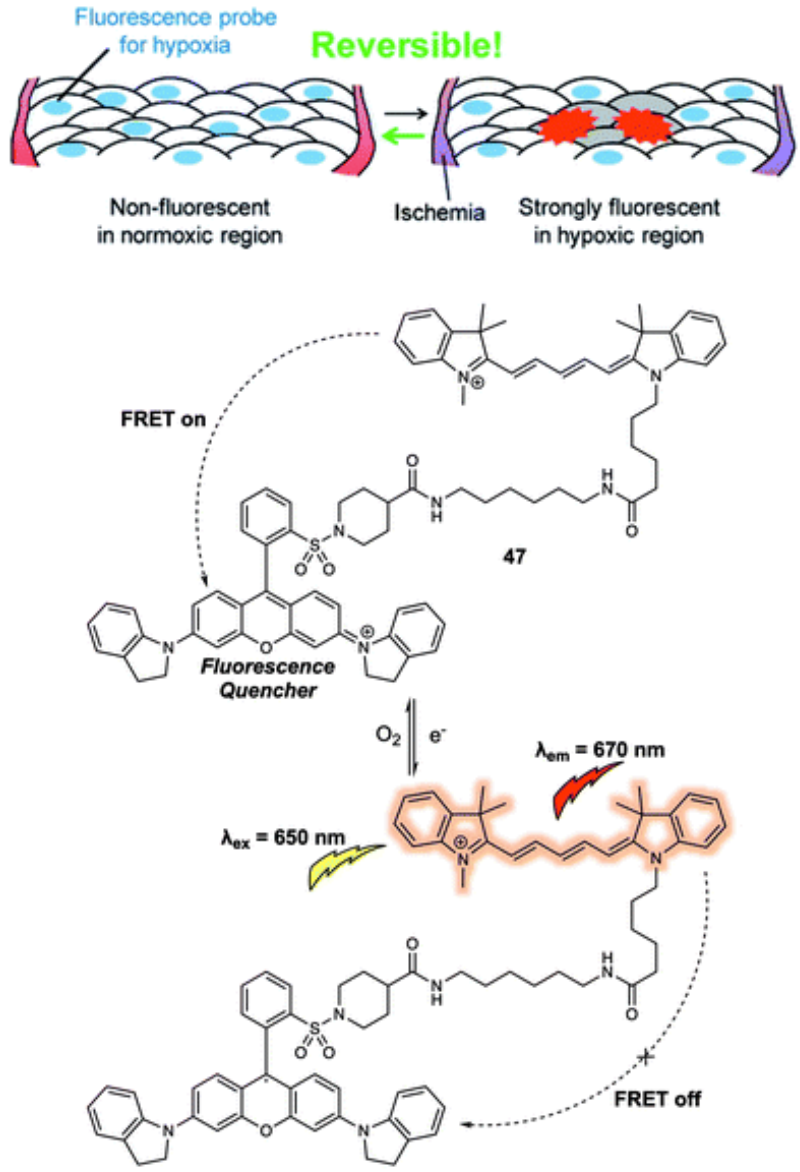


Fig. 29 Design of the FRET-based probe **47** that allowed hypoxia in live cells to be monitored by visual means. Reproduced with permission from ref. 39 (*J. Am. Chem. Soc.*, 2012, **134**, 19588–19591). Copyright (2012) American Chemical Society.

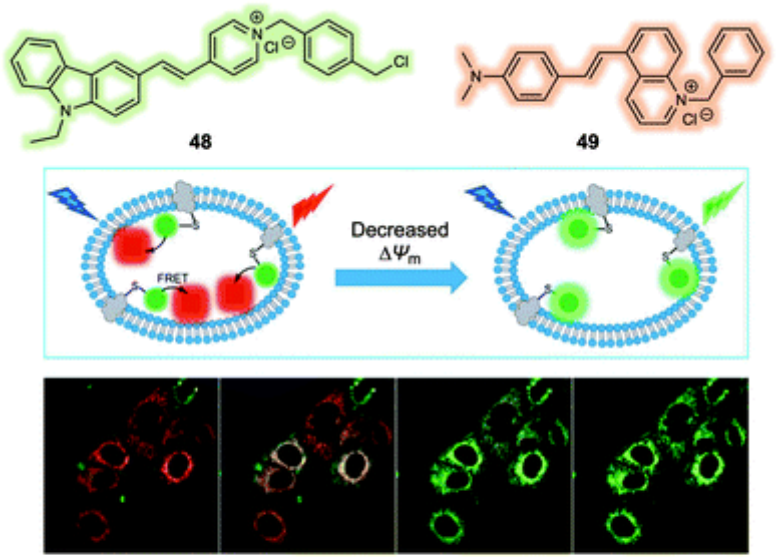
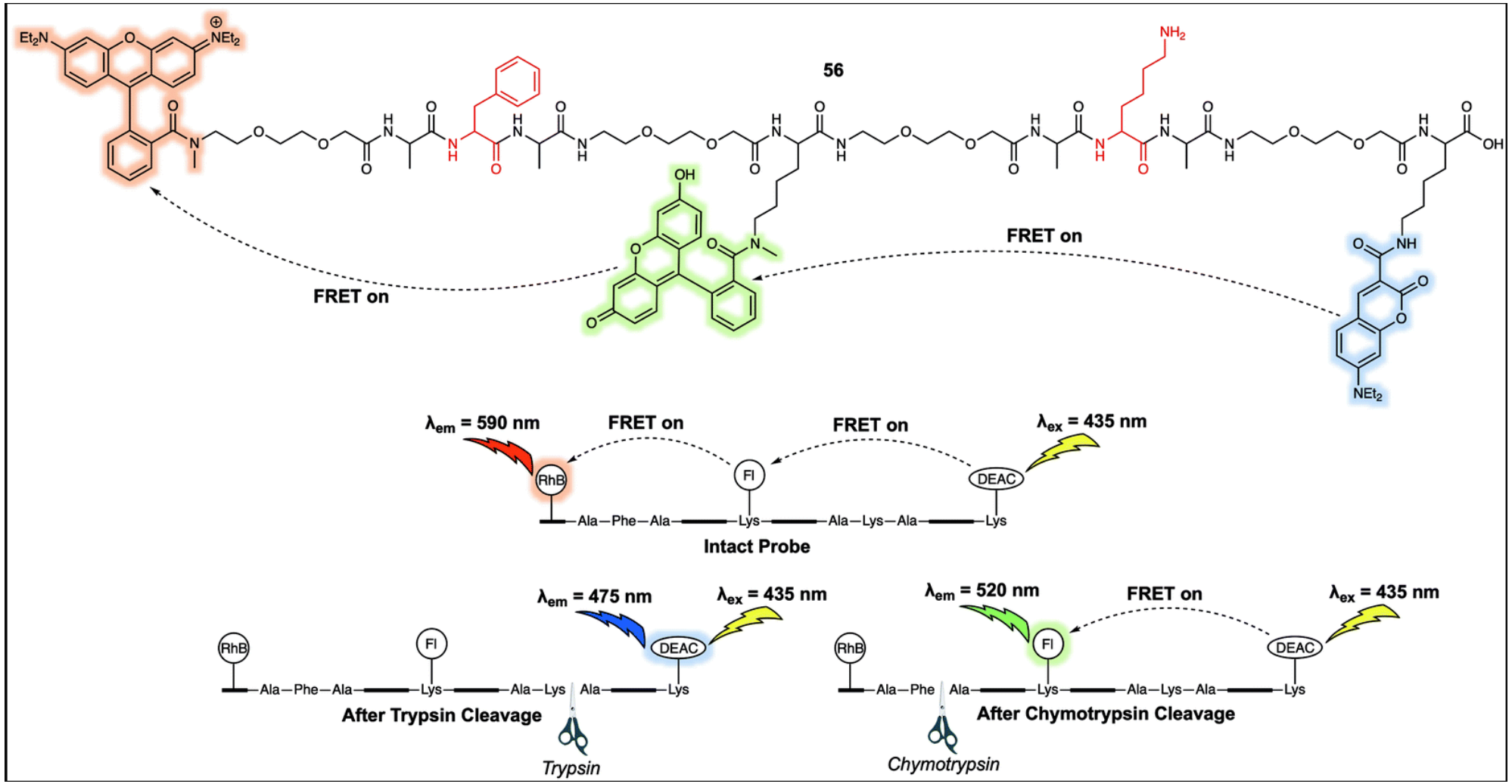
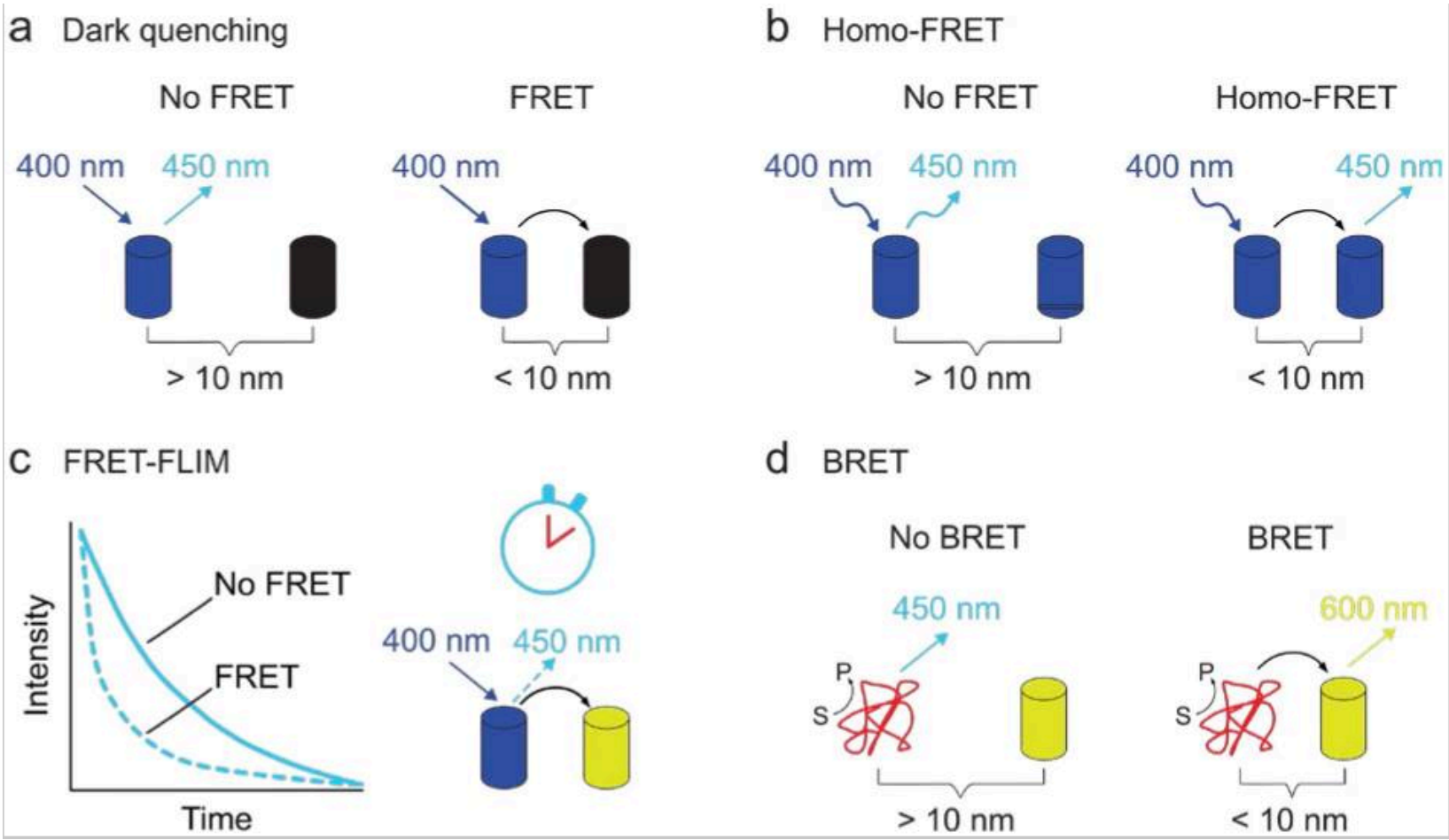


Fig. 30 Design of a FRET system (**48**/**49**) for the ratiometric detection of mitochondria membrane potential ($\Delta\Psi_m$). Reproduced with permission from ref. 40 (*Anal. Chem.*, 2019, **91**, 3704–3709). Copyright (2019) American Chemical Society.

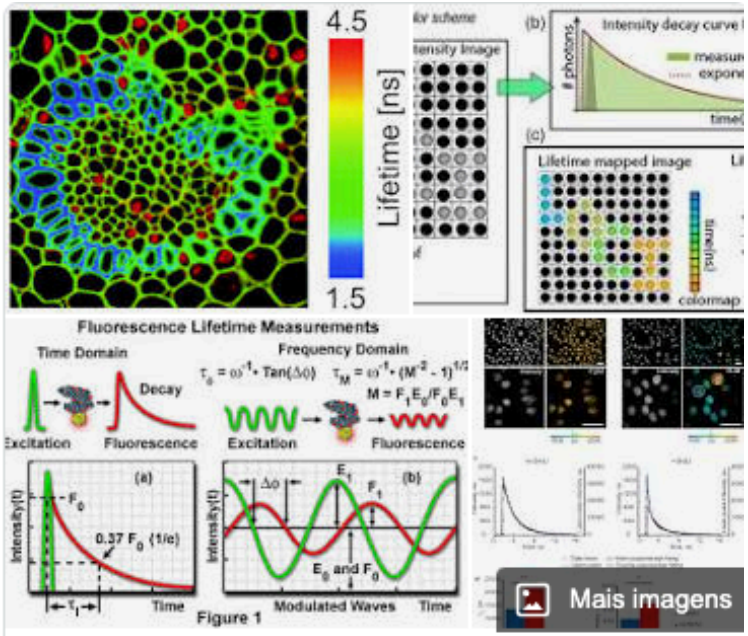


Chemical structure of the dual-responsive, three-fluorophore probe **56** and the working mechanism proposed that allows for the detection of chymotrypsin and trypsin.



Different variations on traditional FRET where: (a) the acceptor releases the obtained energy non-radiatively (dark quenching), (b) the FRET pair consists of identical fluorophores (homo-FRET), (c) the fluorescence lifetim of the donor fluorophore is used to monitor energy transfer (FRET-FLIM), or (d) the donor fluorophore is replaced by a bioluminescent protein (red) to thereby eliminate the need for external illumination (BRET). The depicted excitation and emission wavelengths serve as an example.

https://www.youtube.com/watch?v=DOkHUQi-000



Fluorescence-lifetime imaging microscopy

Traduzido do inglês - A microscopia de imagem de vida útil de fluorescência ou FLIM é uma técnica de imagem baseada nas diferenças na taxa de decaimento exponencial da emissão de fótons de um fluoróforo de uma amostra. Wikipedia (inglês)

Ver descrição original

Fluorescence-lifetime imaging microscopy or FLIM is an imaging technique based on the differences in the exponential decay rate of the photon emission of a fluorophore from a sample. It can be used as an imaging technique in confocal microscopy, two-photon excitation microscopy, and multiphoton tomography. Wikipedia

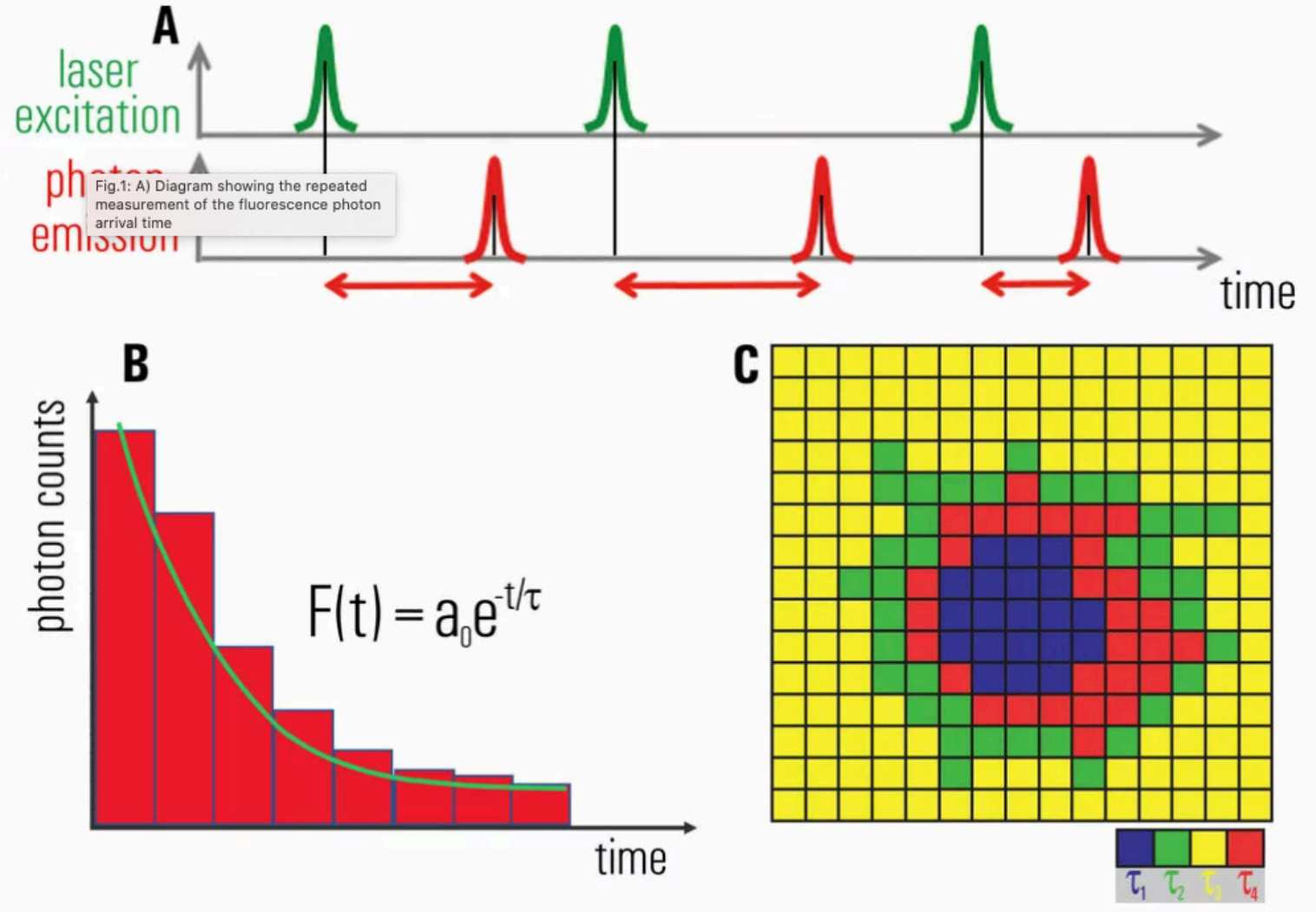
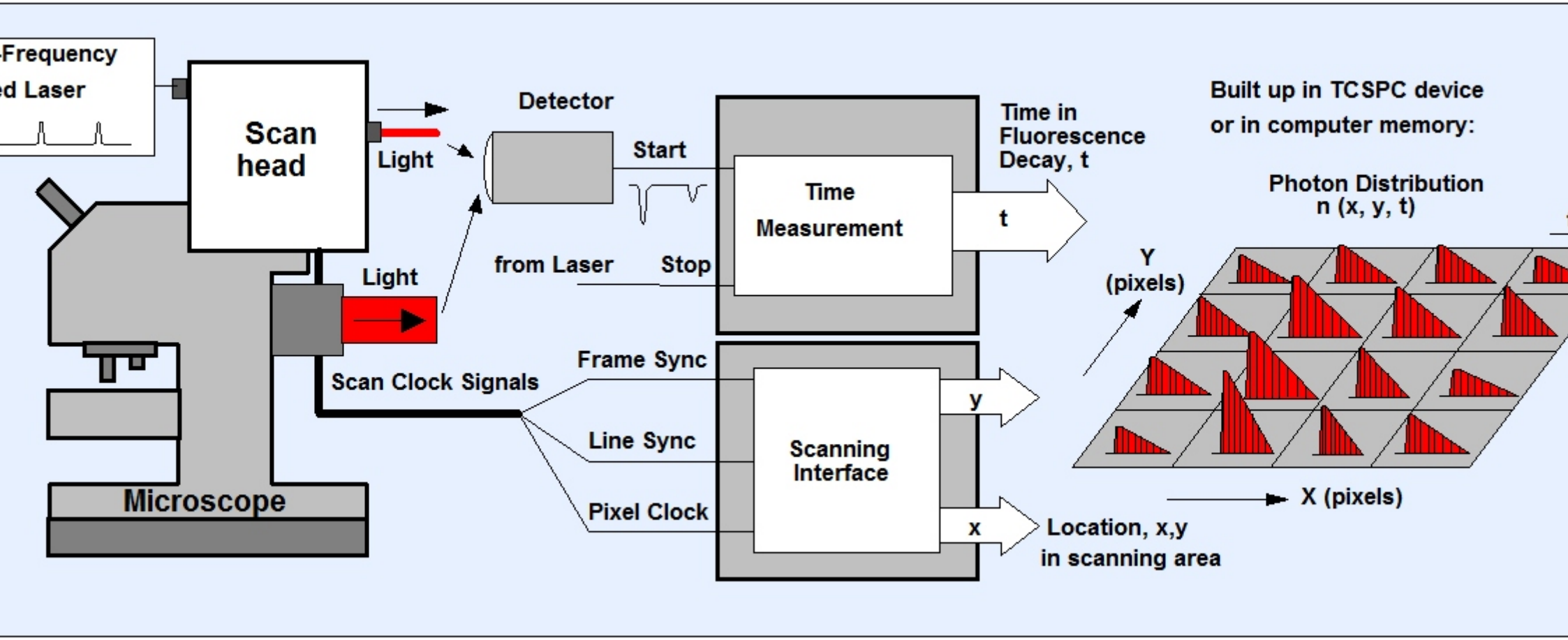
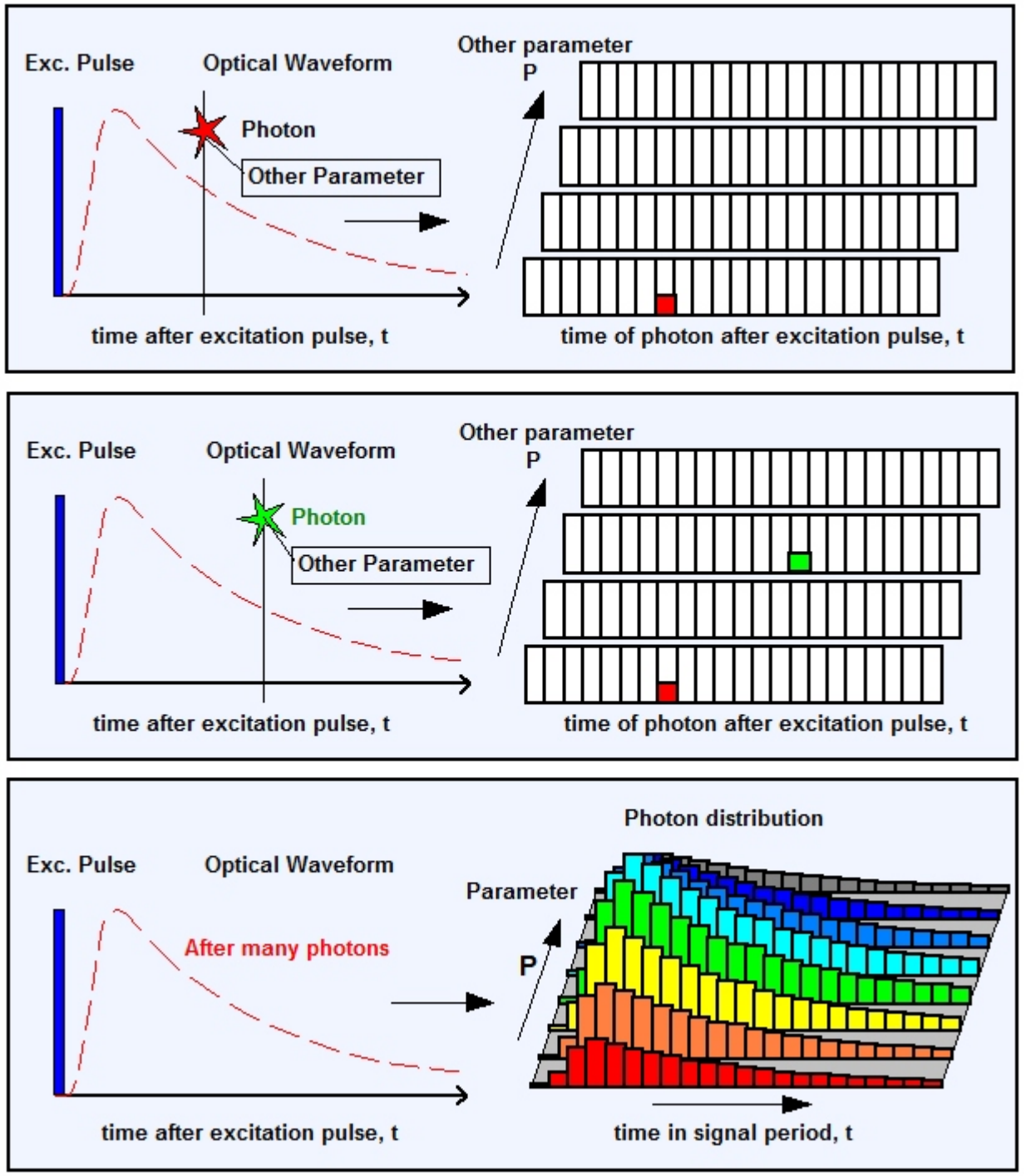
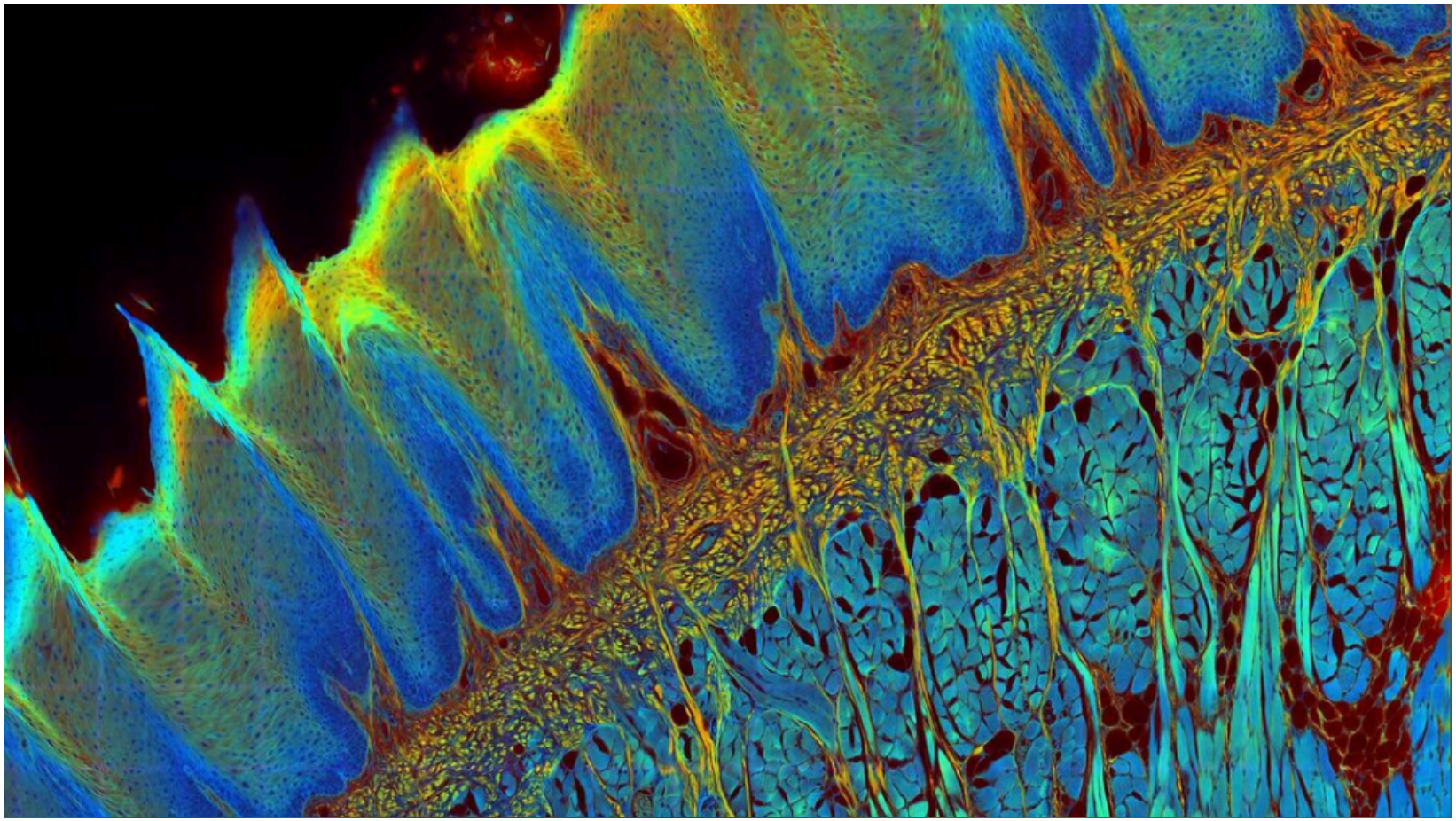
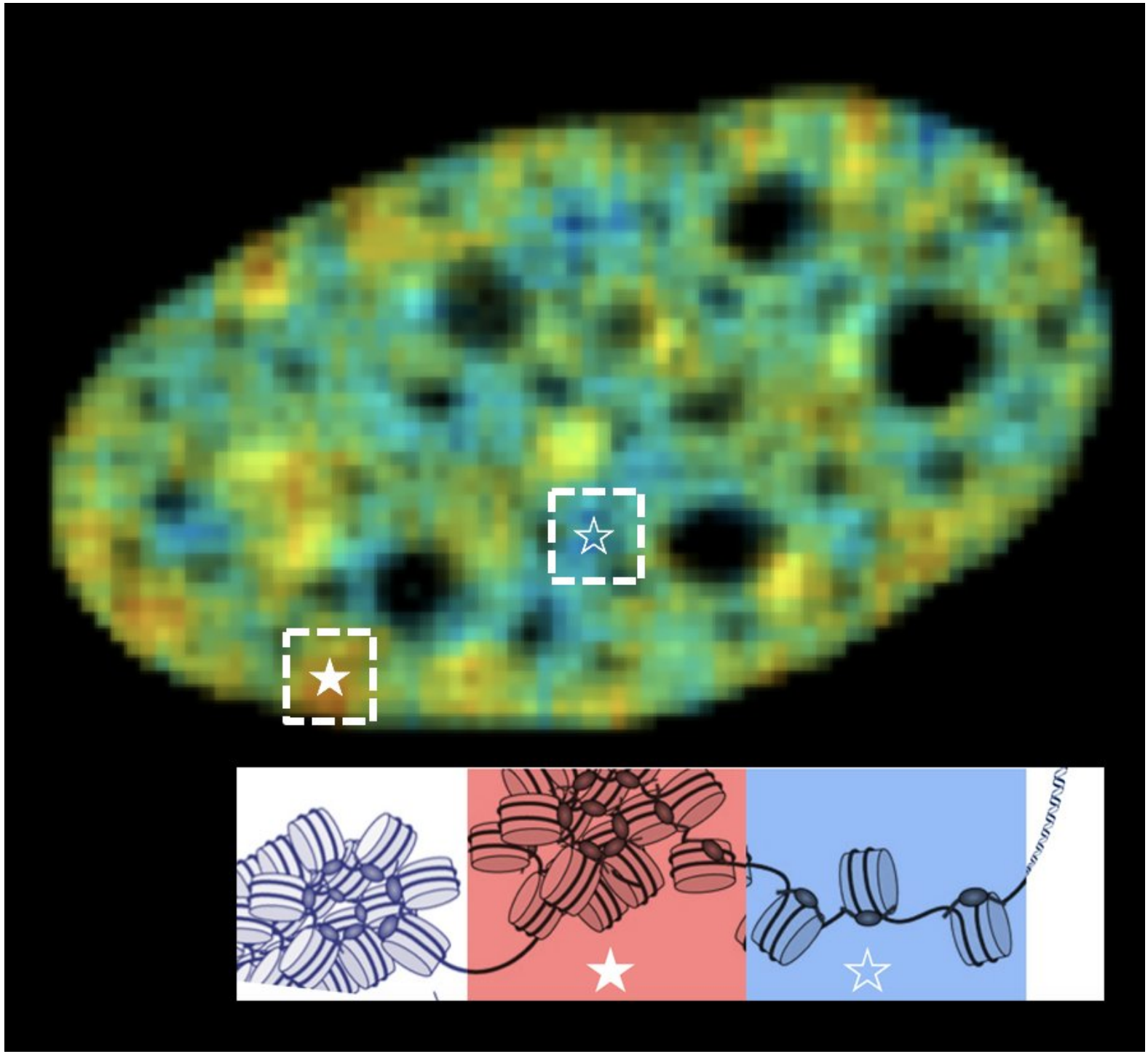


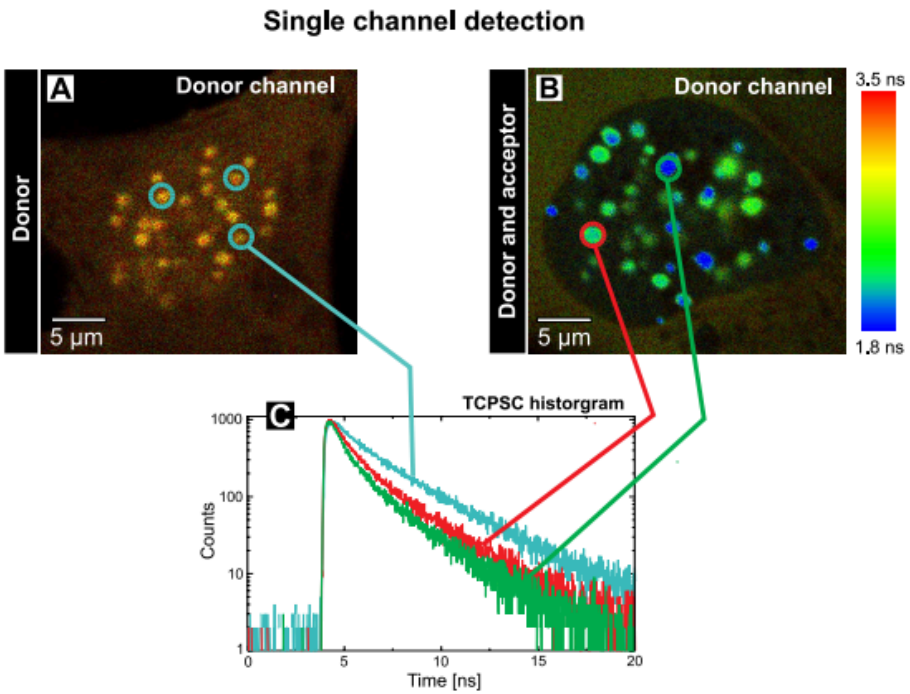
Fig.1: A) Diagram showing the repeated measurement of the fluorescence photon arrival time

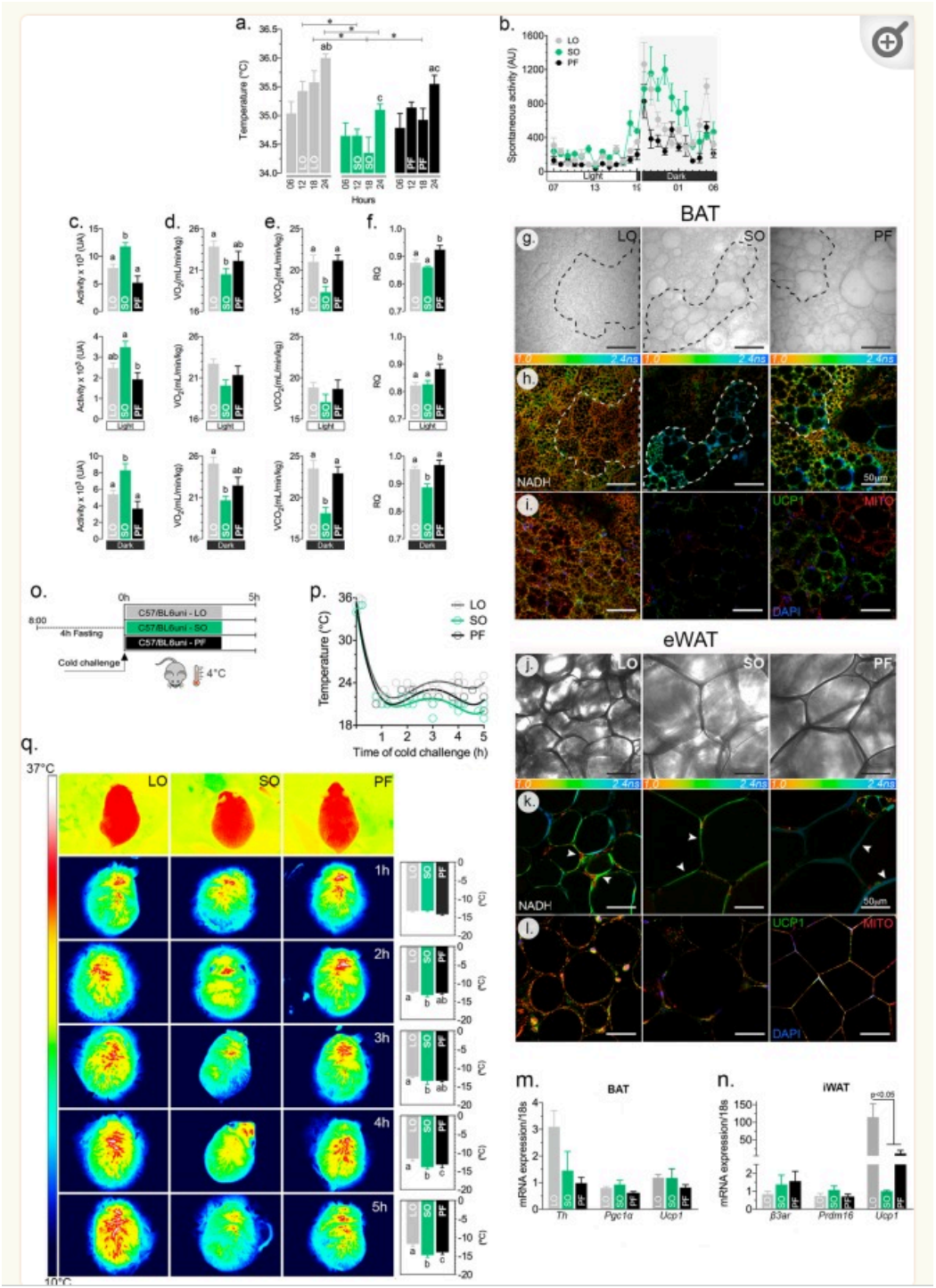

-Frequency
ed Laser
Scan
head
Light
Detector
Start
Time
Measurement
t
Time in
Fluorescence
Decay, t
Built up in TCSPC device
or in computer memory:
Photon Distribution
n (x, y, t)
Light
from Laser
Stop
Y
(pixels)
Frame Sync
Scan Clock Signals
Line Sync
Scanning
Interface
y
Pixel Clock
x
Location, x,y
in scanning area
X (pixels)
Microscope


Exc. Pulse
Optical Waveform
Photon
Other Parameter
time after excitation pulse, t
Other parameter
P
time of photon after excitation pulse, t
Exc. Pulse
Optical Waveform
Photon
Other Parameter
time after excitation pulse, t
Other parameter
P
time of photon after excitation pulse, t
Exc. Pulse
Optical Waveform
After many photons
time after excitation pulse, t
Photon distribution
Parameter
P
time in signal period, t

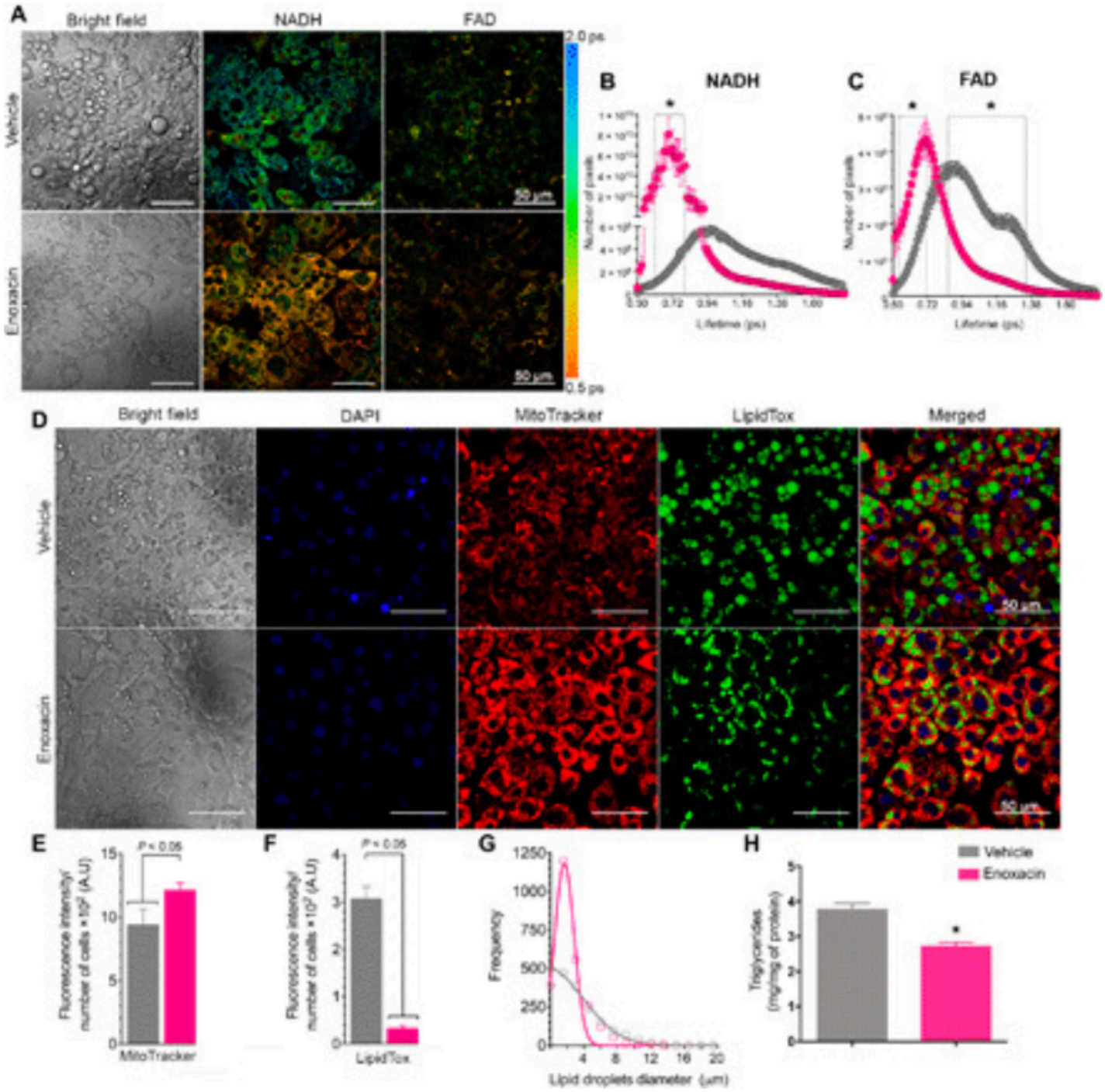




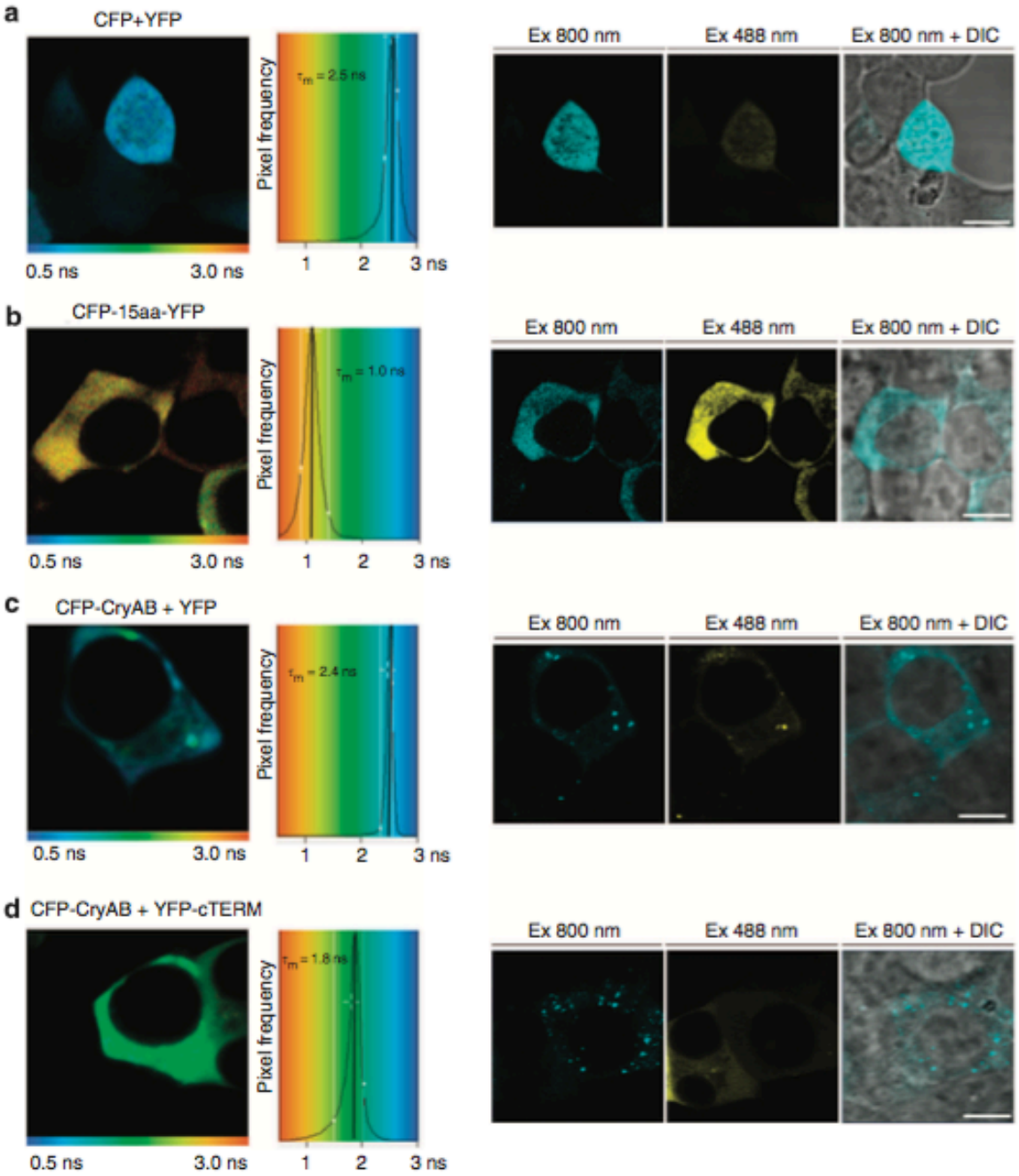

Single channel detection
A
Donor channel
Donor
5 µm
B
Donor channel
Donor and acceptor
5 µm
3.5 ns
1.8 ns
C
TCPSC historgram
Counts
Time [ns]
Dual channel detection
D
Donor channel
Donor and acceptor
1
2
5 µm
E
FRET channel
Donor and acceptor
1
2
5 µm
3.5 ns
1.8 ns
F
TCPSC histogram
Counts
Time [ns]
G
TCPSC histogram
1000
Counts
Time [ns]



Ferrucci et al. Sci Rep 2019



Rocha et al. 20


a
CFP+YFP
Pixel frequency
τ_m = 2.5 ns
0.5 ns
3.0 ns
1
2
3 ns
Ex 800 nm
Ex 488 nm
Ex 800 nm + DIC
b
CFP-15aa-YFP
τ_m = 1.0 ns
c
CFP-CryAB + YFP
τ_m = 2.4 ns
d
CFP-CryAB + YFP-cTERM
τ_m = 1.8 ns