



THE UNIVERSITY of EDINBURGH
Centre for Inflammation Research



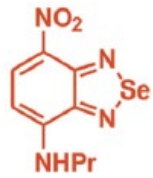
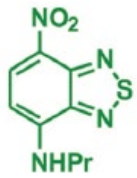
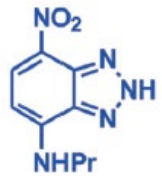
Seeing is believing: organic fluorophores for imaging inside and outside humans

Prof Marc Vendrell

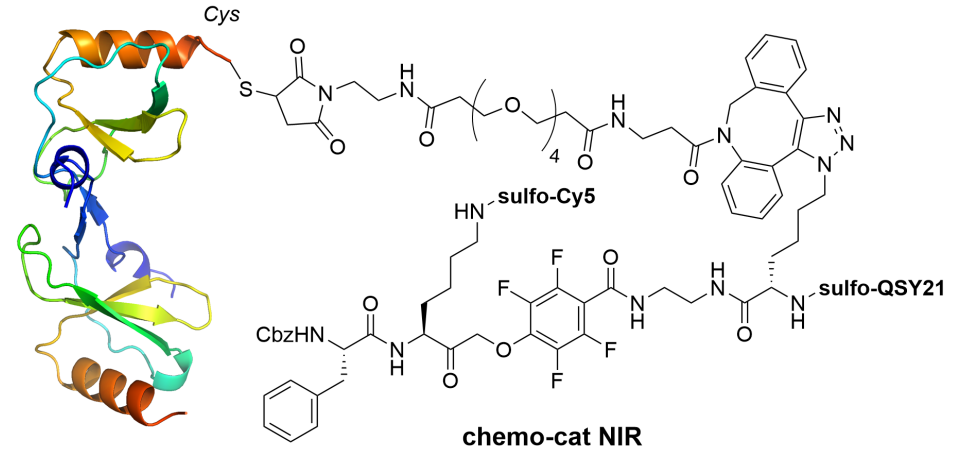
Chair of Translational Chemistry and
co-Head of IRR Chemistry Hub
The University of Edinburgh

IASOC Sep 2024 - Ischia

Imaging tool builders



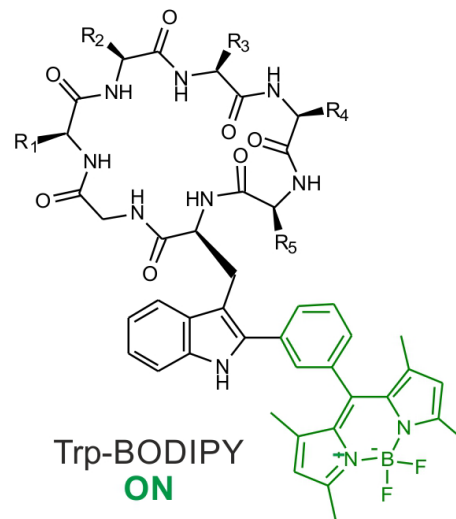
*small
fluorophores*



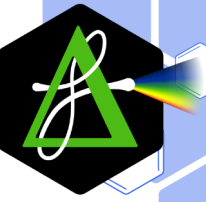
*fluorescent
peptides*

molecular
weight

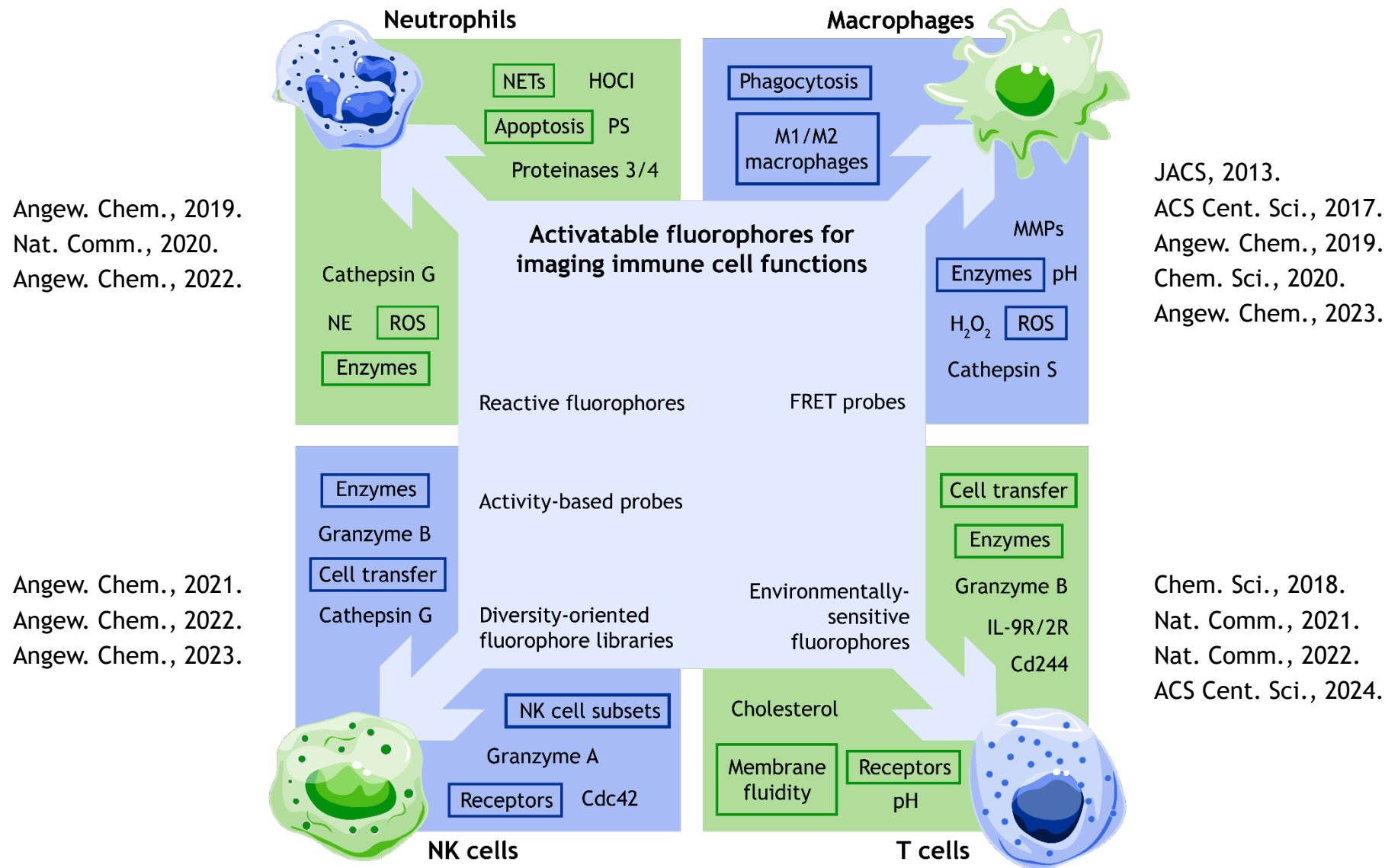
*fluorescent
amino acids*



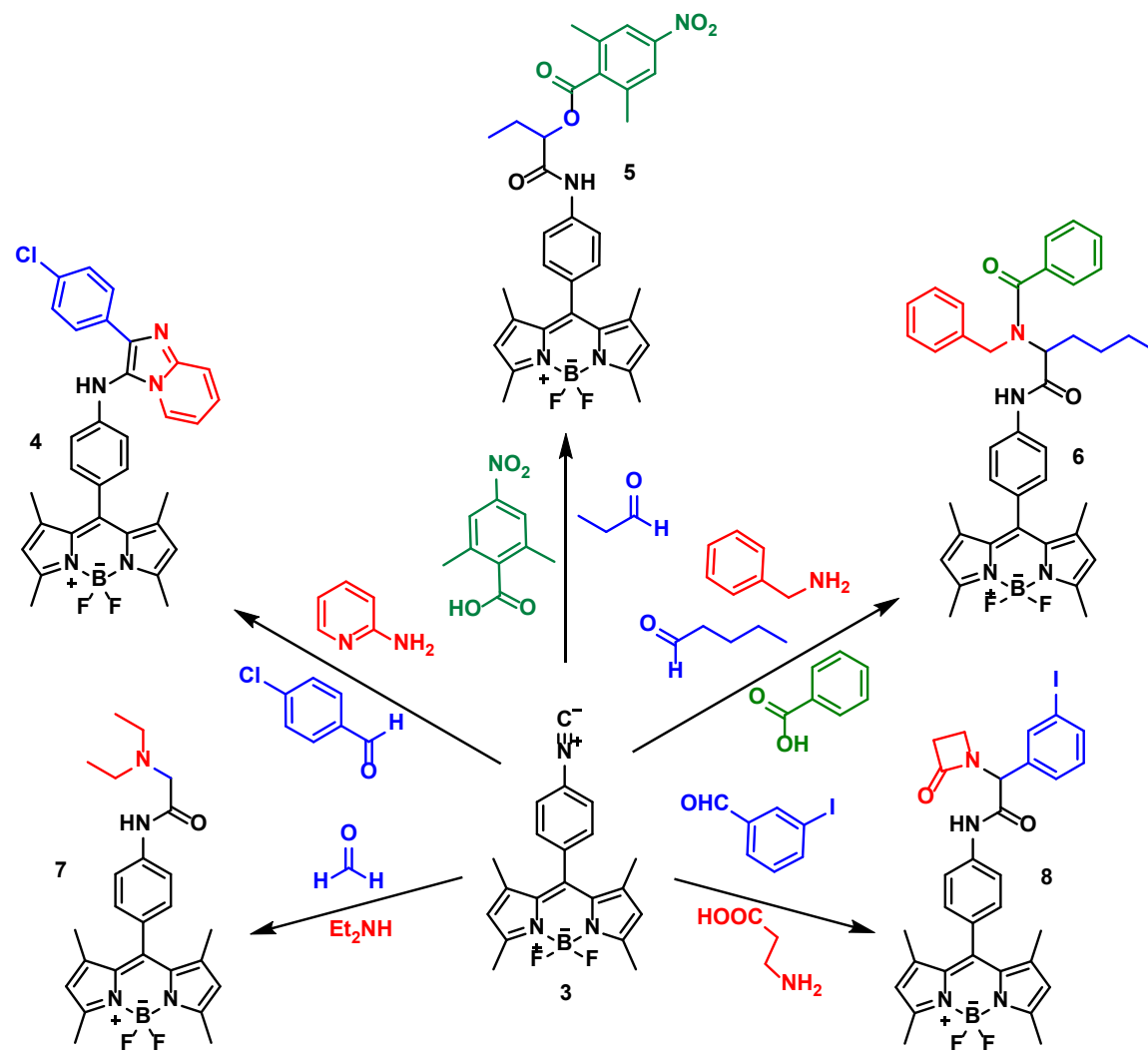
*fluorescent
proteins*



Imaging inflammation and immune cell function



Small-molecule fluorophores

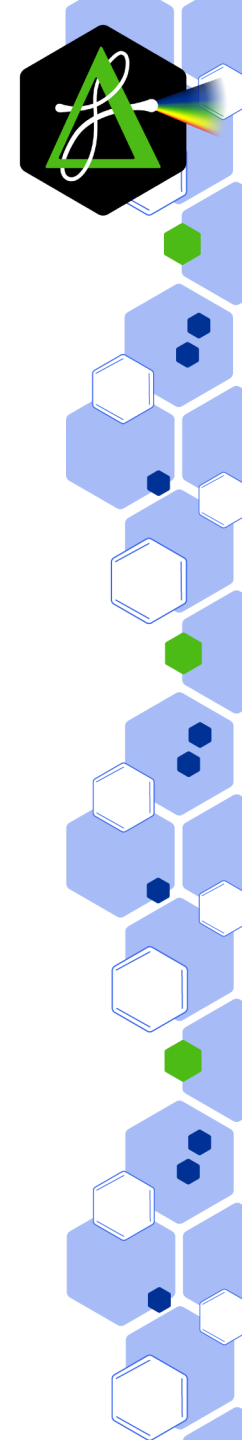
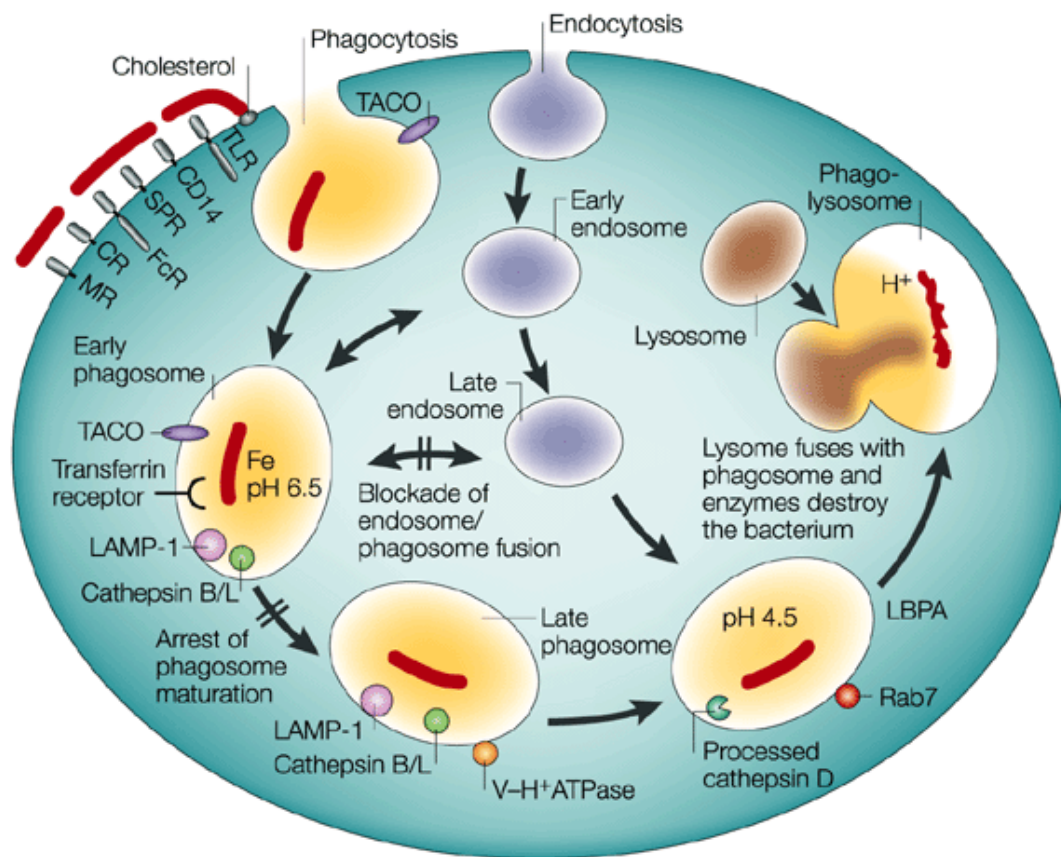


Vazquez-Romero et al. *JACS*, 2013, 16018.

Collaboration with Rodolfo Lavilla (previous work: Arevalo et al. *EuJOC*, 2009, 617; Preciado et al. *Angew.*, 2012, 6874).

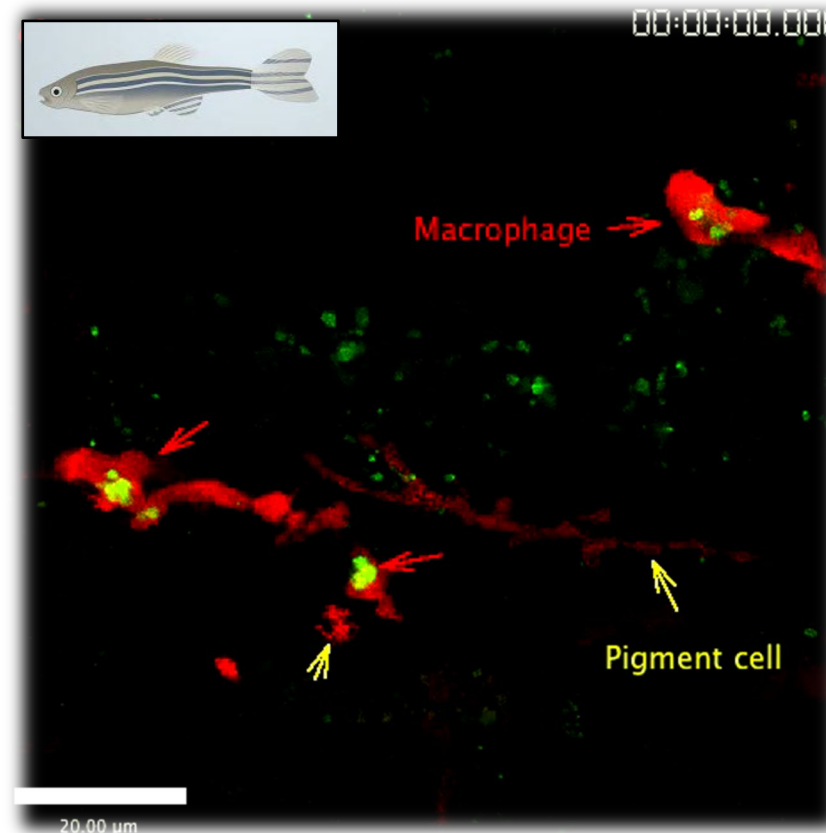
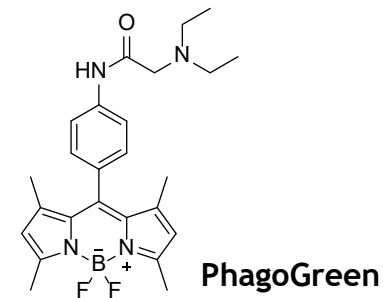
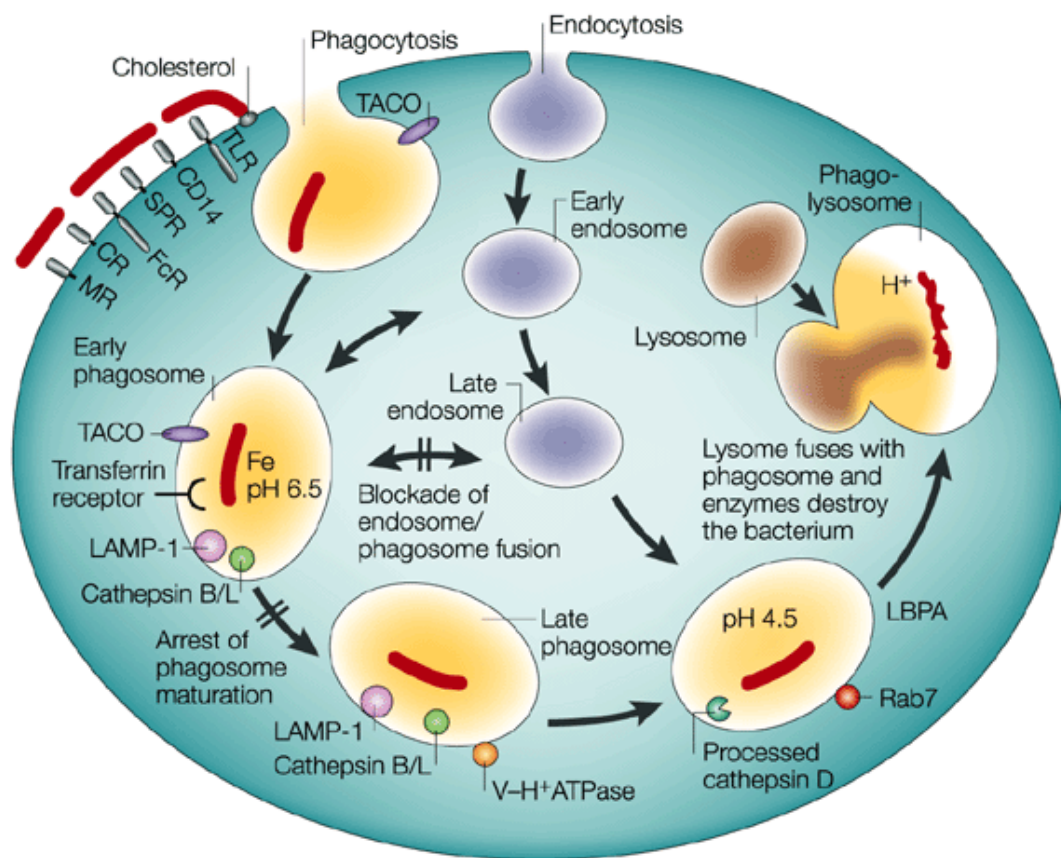
Small-molecule fluorophores

Phagosomal acidification in macrophages



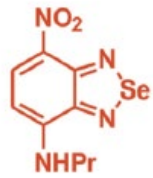
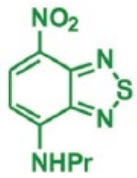
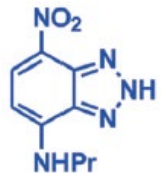
Small-molecule fluorophores

Phagosomal acidification in macrophages

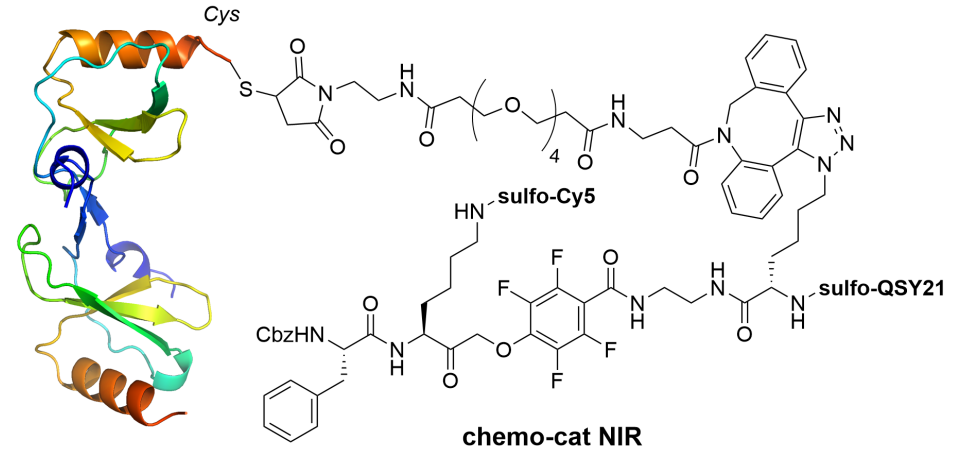


Collaboration with Yi Feng
(Feng et al. *Nature*, 2009, 921;
PLoS Biol., 2010, e1000562)

Imaging tool builders

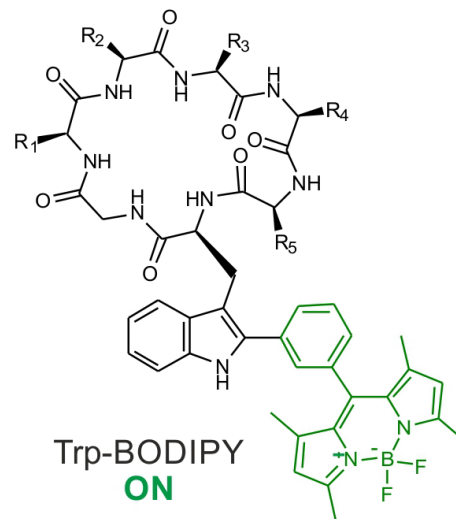


*small
fluorophores*



*fluorescent
peptides*

*fluorescent
amino acids*

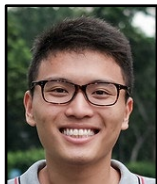
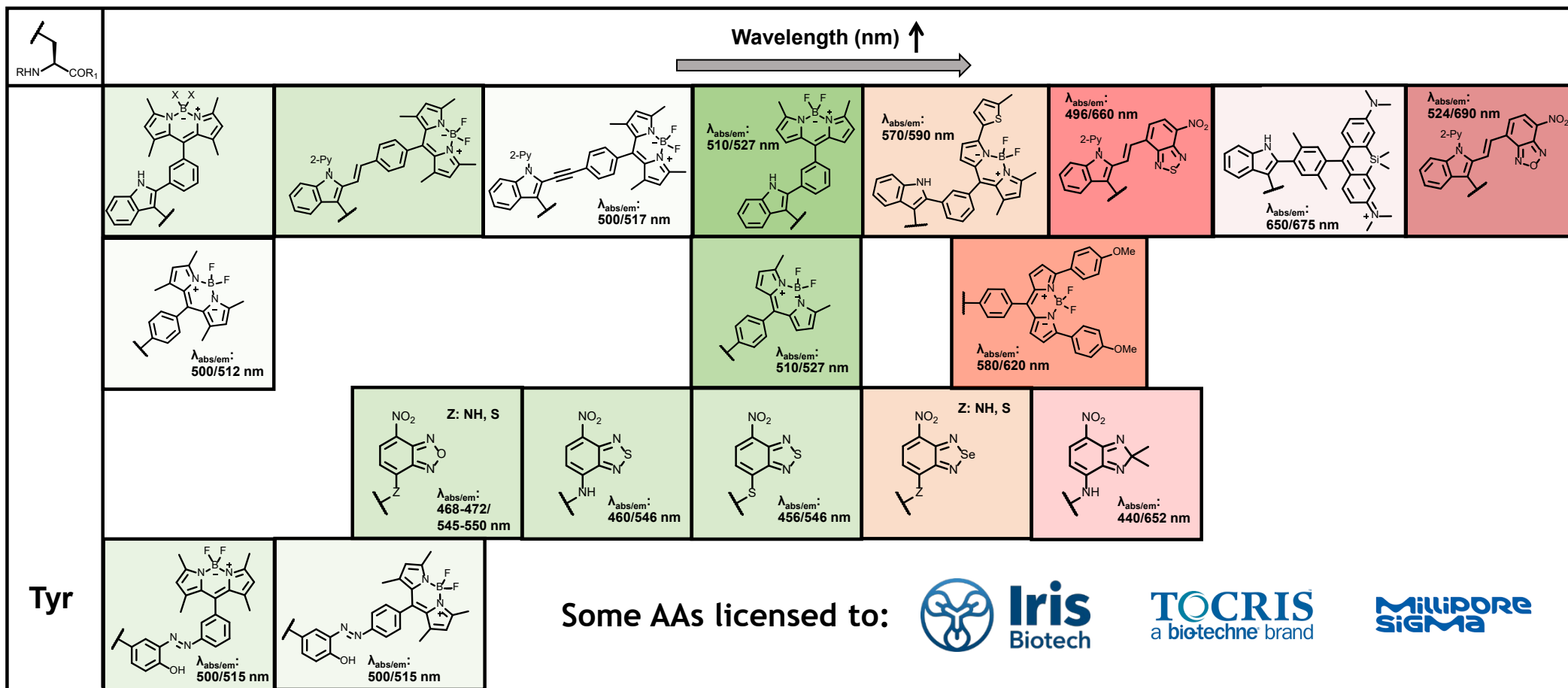
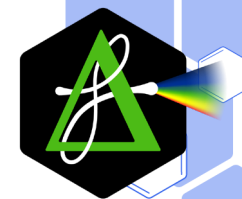


*fluorescent
proteins*

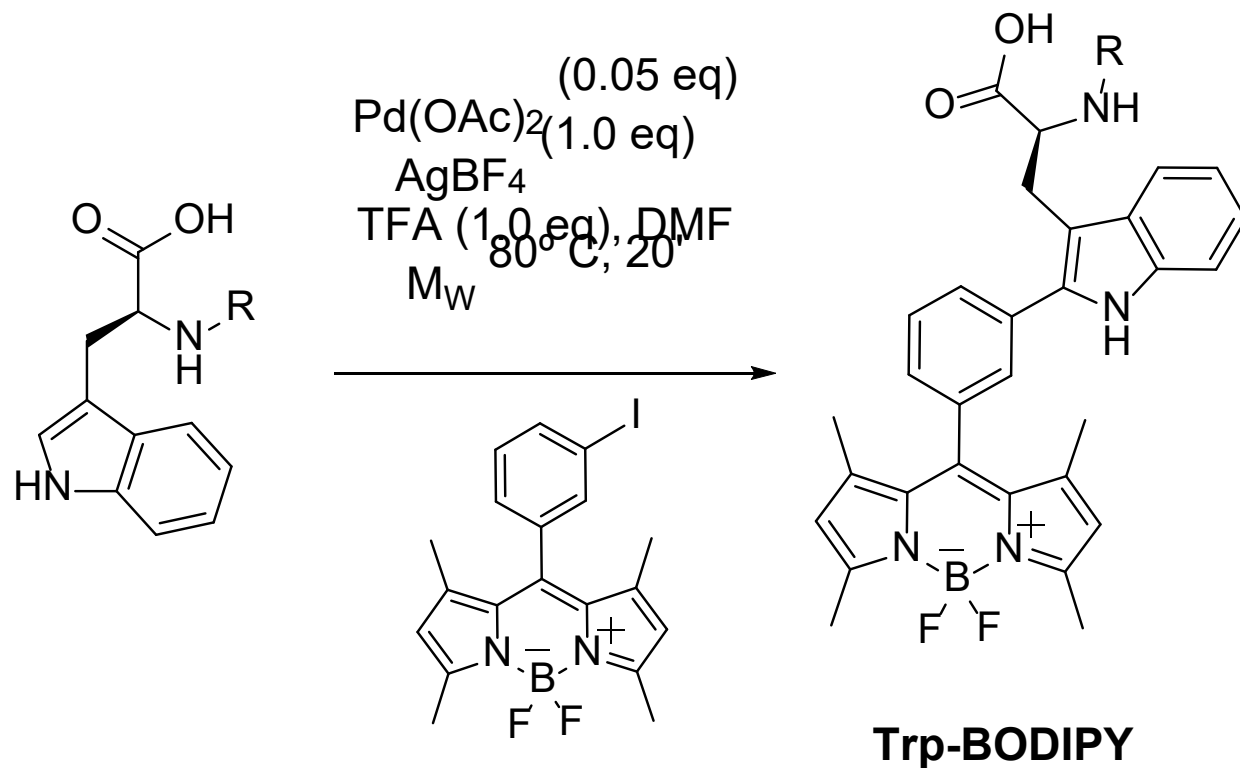
molecular
weight



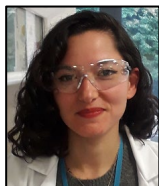
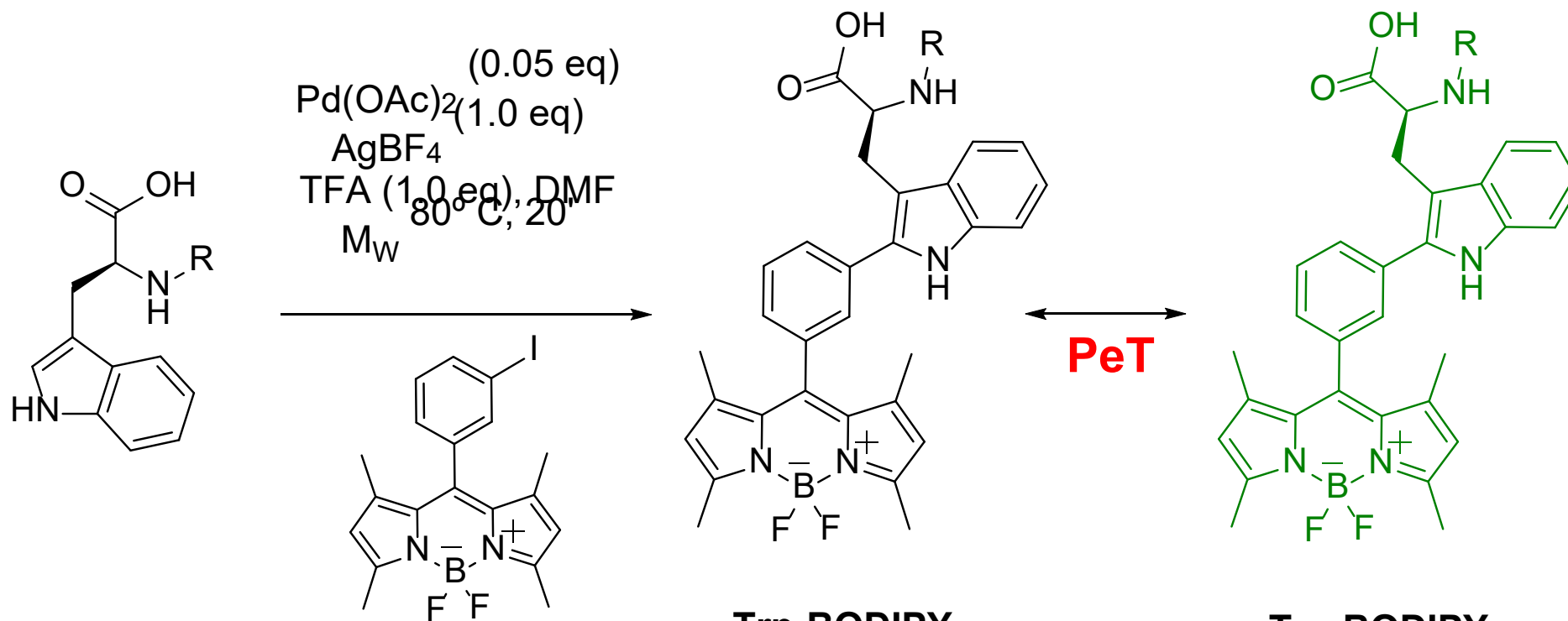
Fluorescent amino acids



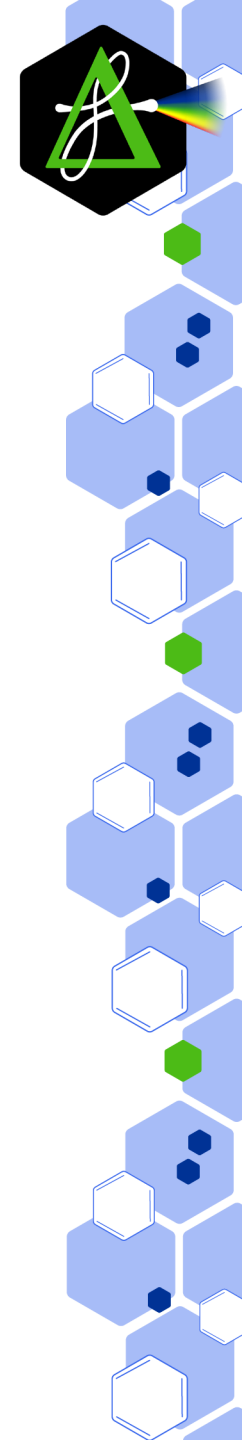
Fluorogenic (OFF-to-ON) amino acids



Fluorogenic (OFF-to-ON) amino acids



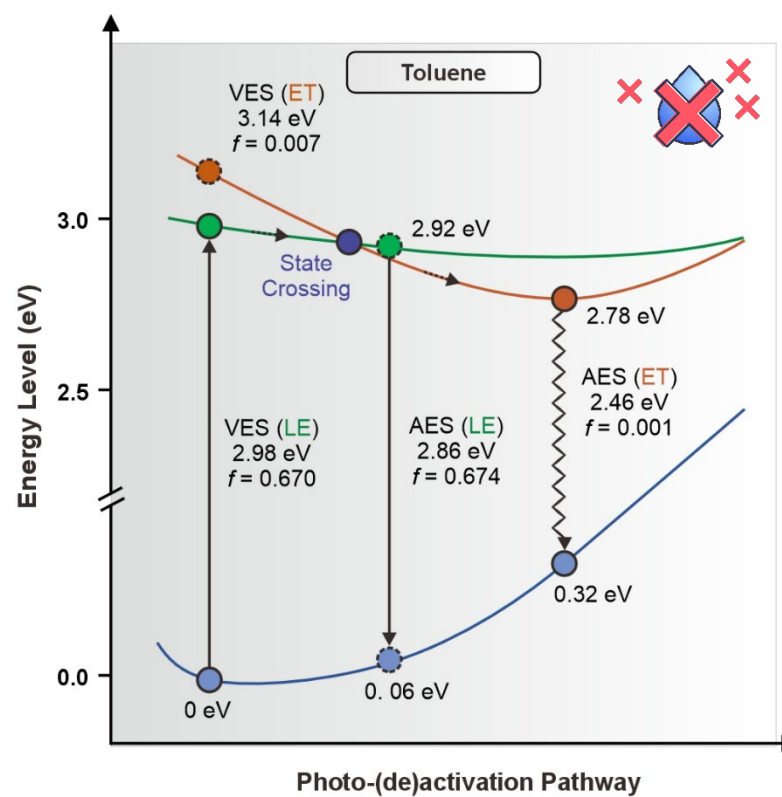
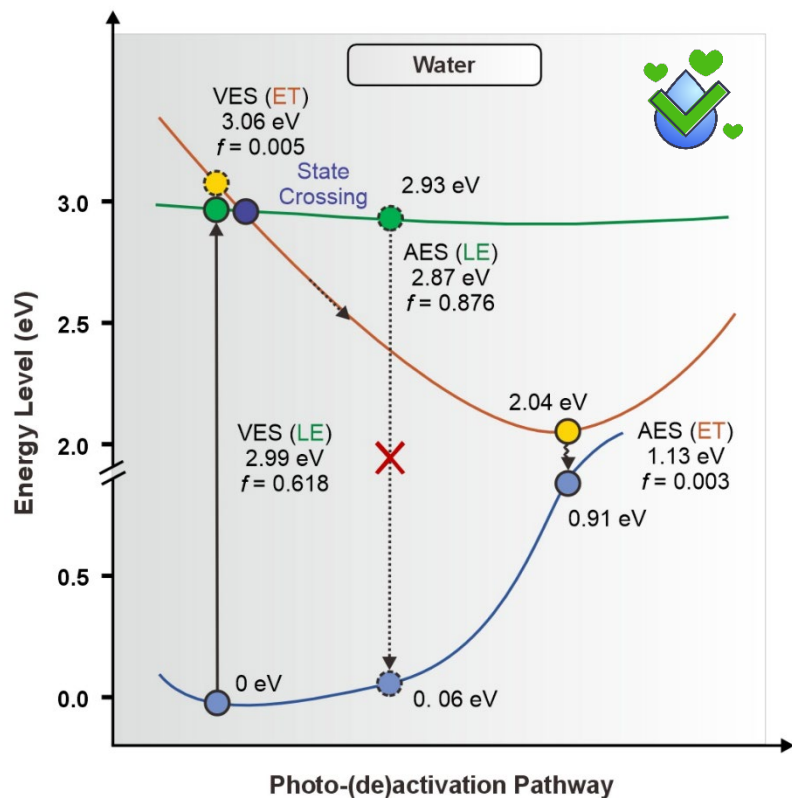
Mendive-Tapia et al. *Nat. Commun.*, 2016, 10940.
and *Nat. Protocols*, 2017, 1588.



Fluorogenic (OFF-to-ON) amino acids

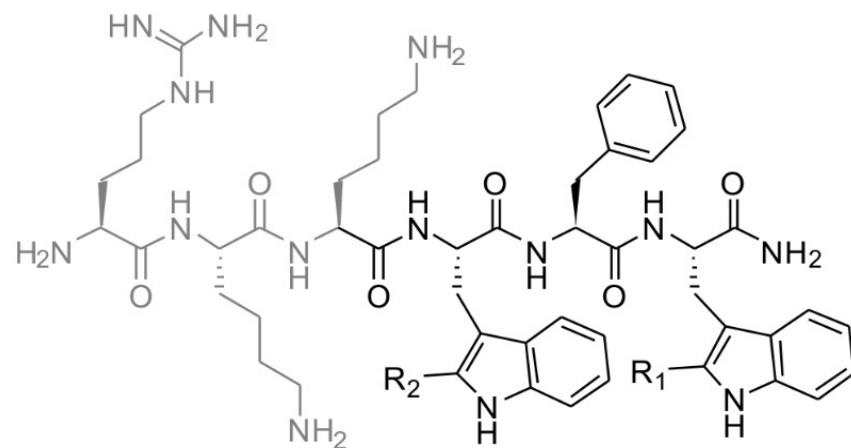


Photoinduced electron transfer (PeT) in Trp-BODIPY



Fluorogenic (OFF-to-ON) peptides: case 1

Antifungal peptides



hydrophilic domain

hydrophobic domain

R₁, R₂: H (4)

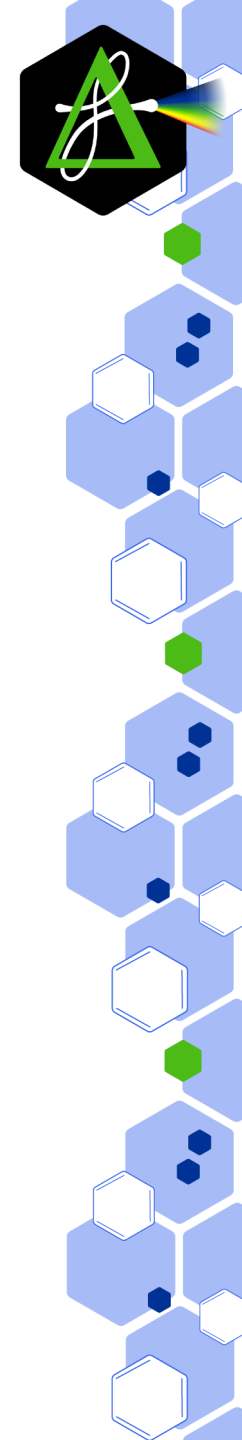
R₁: H, R₂: BODIPY (5)

R₁: BODIPY, R₂: H (6)

R₁, R₂: BODIPY (7)

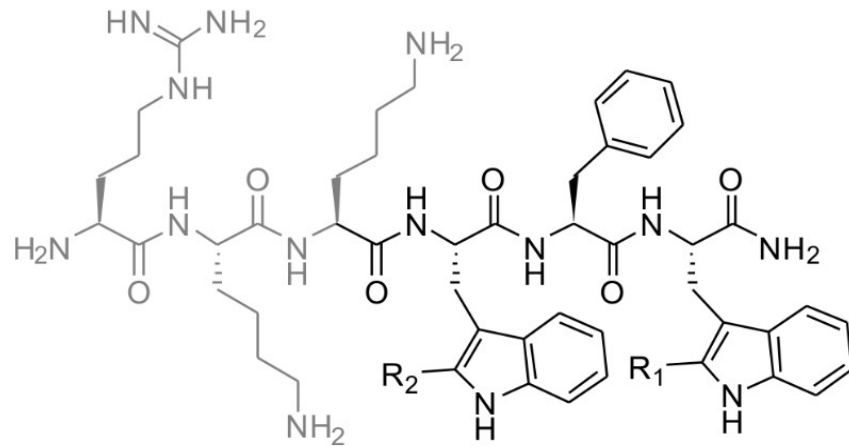


Can Zhao (with Prof. Nick Read)



Fluorogenic (OFF-to-ON) peptides: case 1

Antifungal peptides

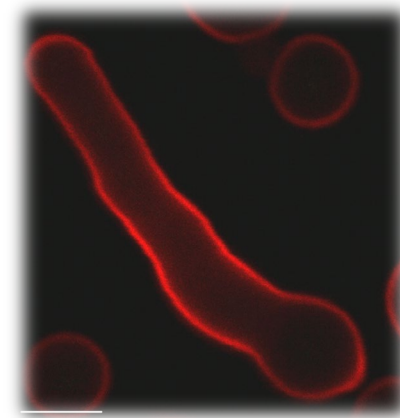


hydrophilic domain

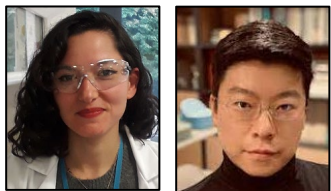
hydrophobic domain

- R₁, R₂: H (4)
- R₁: H, R₂: BODIPY (5)
- R₁: BODIPY, R₂: H (6)
- R₁, R₂: BODIPY (7)

	<i>Aspergillus fumigatus</i> [1]	<i>Klebsiella pneumoniae</i> [2]	<i>Escherichia coli</i> [2]	<i>Pseudomonas aeruginosa</i> [2]	Human RBCs[3]
4	7.9 ± 0.2	93%	>99%	>99%	99%
5	3.6 ± 0.1	87%	94%	>99%	>99%
6	3.0 ± 0.1	96%	95%	>99%	>99%
7	2.5 ± 0.1	93%	95%	>99%	96%
8	2.0 ± 0.1	94%	96%	>99%	>99%

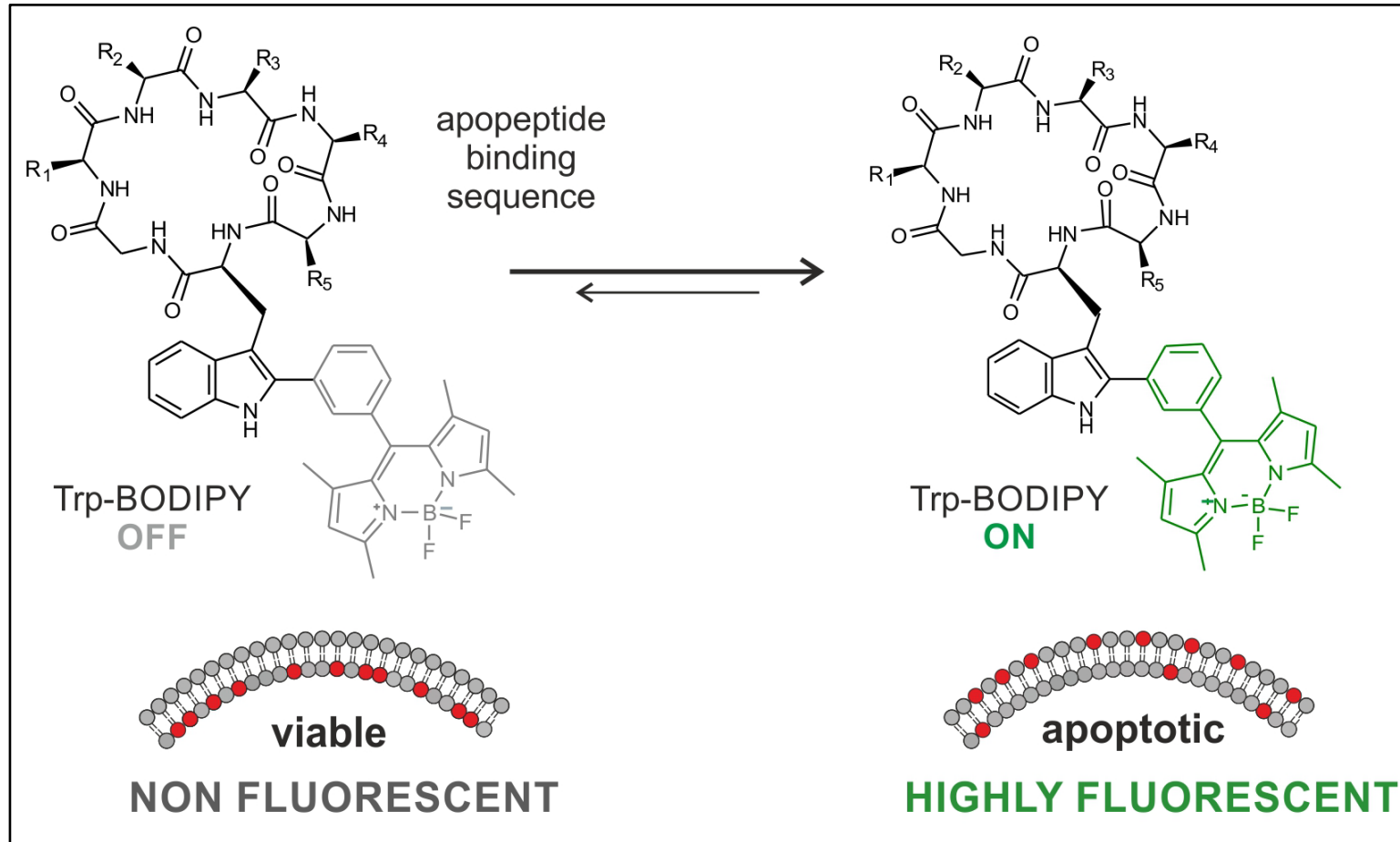


wash-free imaging of live *Aspergillus fumigatus*
(peptide 8 + cell membrane stain)



Can Zhao (with Prof. Nick Read)

Fluorogenic (OFF-to-ON) peptides: case 2

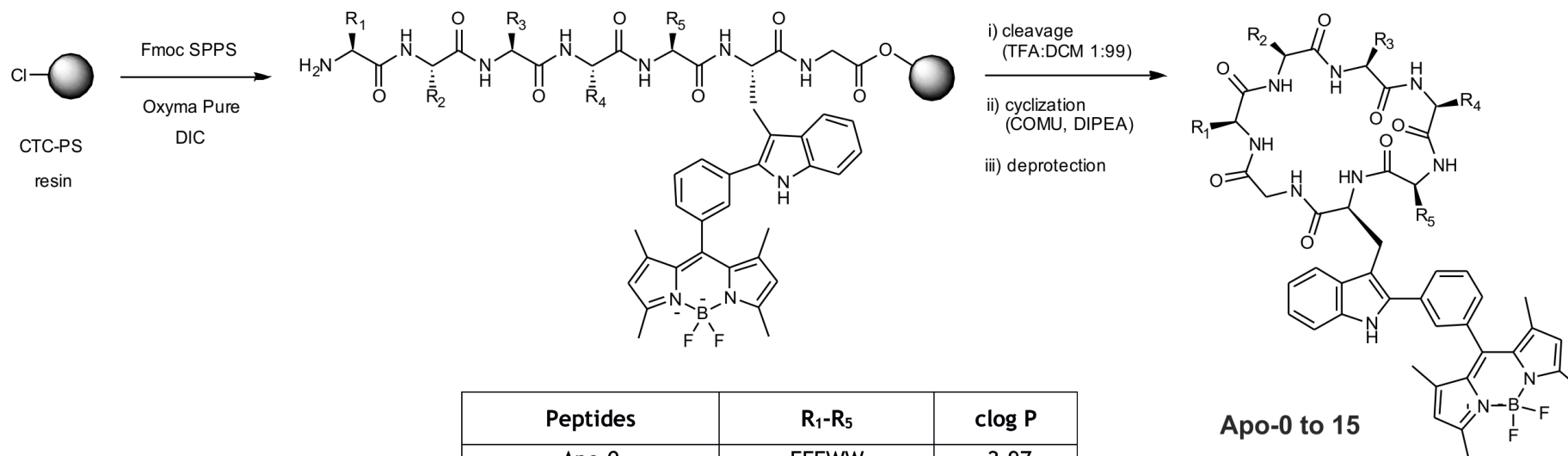


Barth et al. *Nat. Commun.*, 2020, 4027.

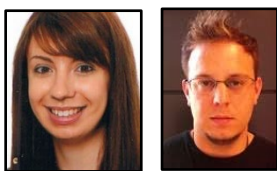
Collaboration with Ian Dransfield (previous work on the 'phagocytic synapse': Barth et al. *Front. Immunol.*, 2017, 01708).



Fluorogenic (OFF-to-ON) peptides: case 2



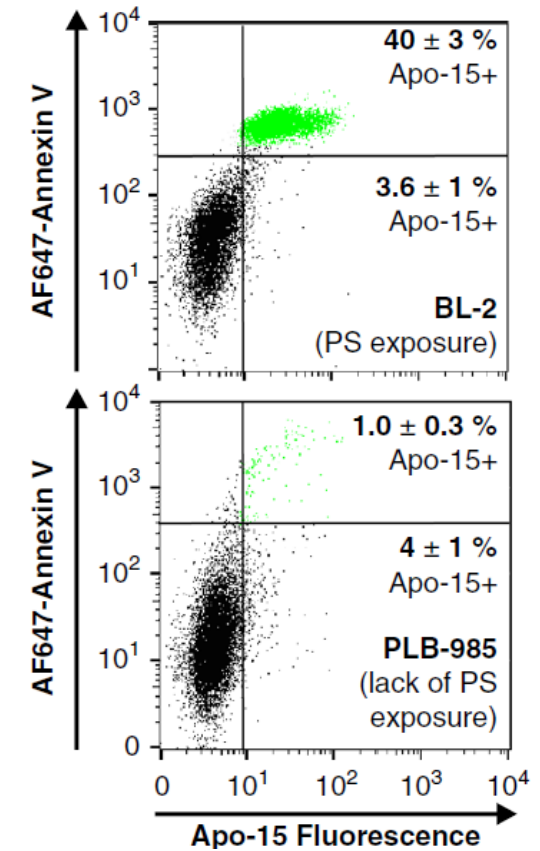
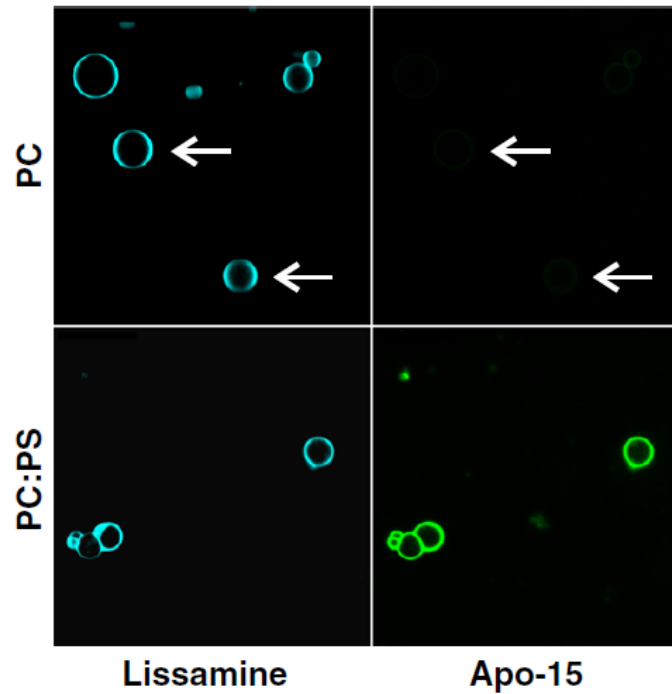
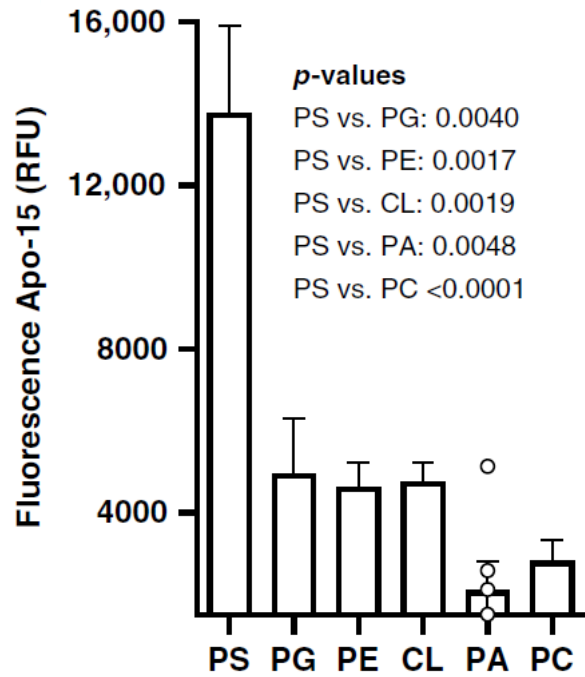
Peptides	R ₁ -R ₅	clog P
Apo-0	EEEWW	-2.97
Apo-2	KKKWW	-2.78
Apo-3	KKKWF	-2.93
Apo-4	KKKFF	-3.08
Apo-5	KWKWK	-2.78
Apo-6	KKWWK	-2.78
Apo-7	WWKKK	-2.78
Apo-8	KWWKK	-2.78
Apo-9	KKKLI	-3.41
Apo-10	KKKVI	-3.94
Apo-11	KKWWW	-0.68
Apo-12	KWWWW	1.43
Apo-13	KKKKW	-4.63
Apo-14	KKKKK	-5.38
Apo-15	RKKWF	-3.38



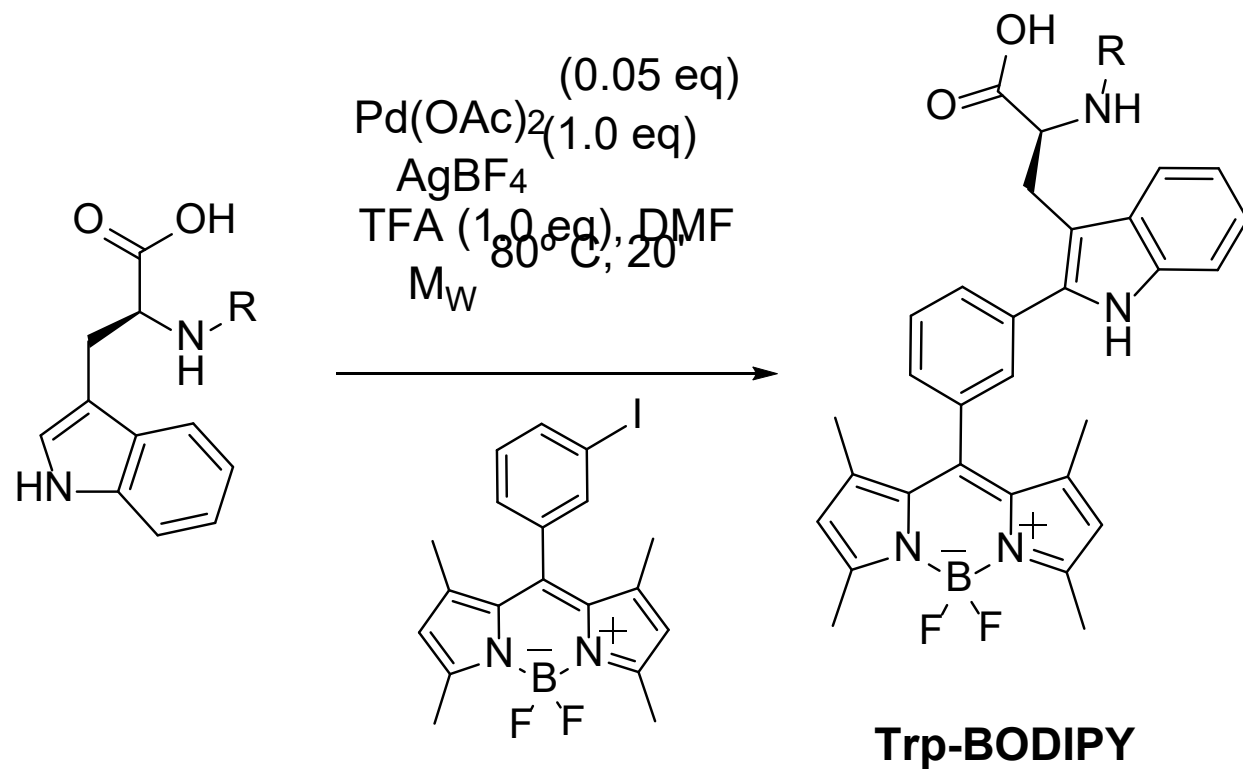
Ramon Subiros-Funosas

Fluorogenic (OFF-to-ON) peptides: case 2

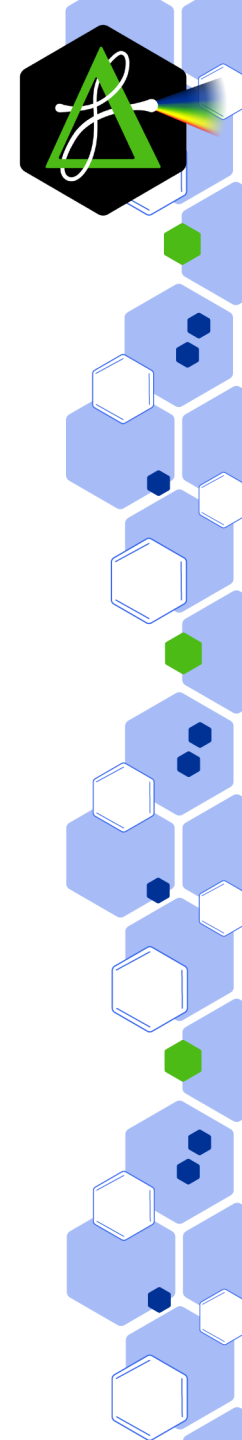
Apo-15 binds to phosphatidylserine in apoptotic cells



Fluorogenic (OFF-to-ON) amino acids

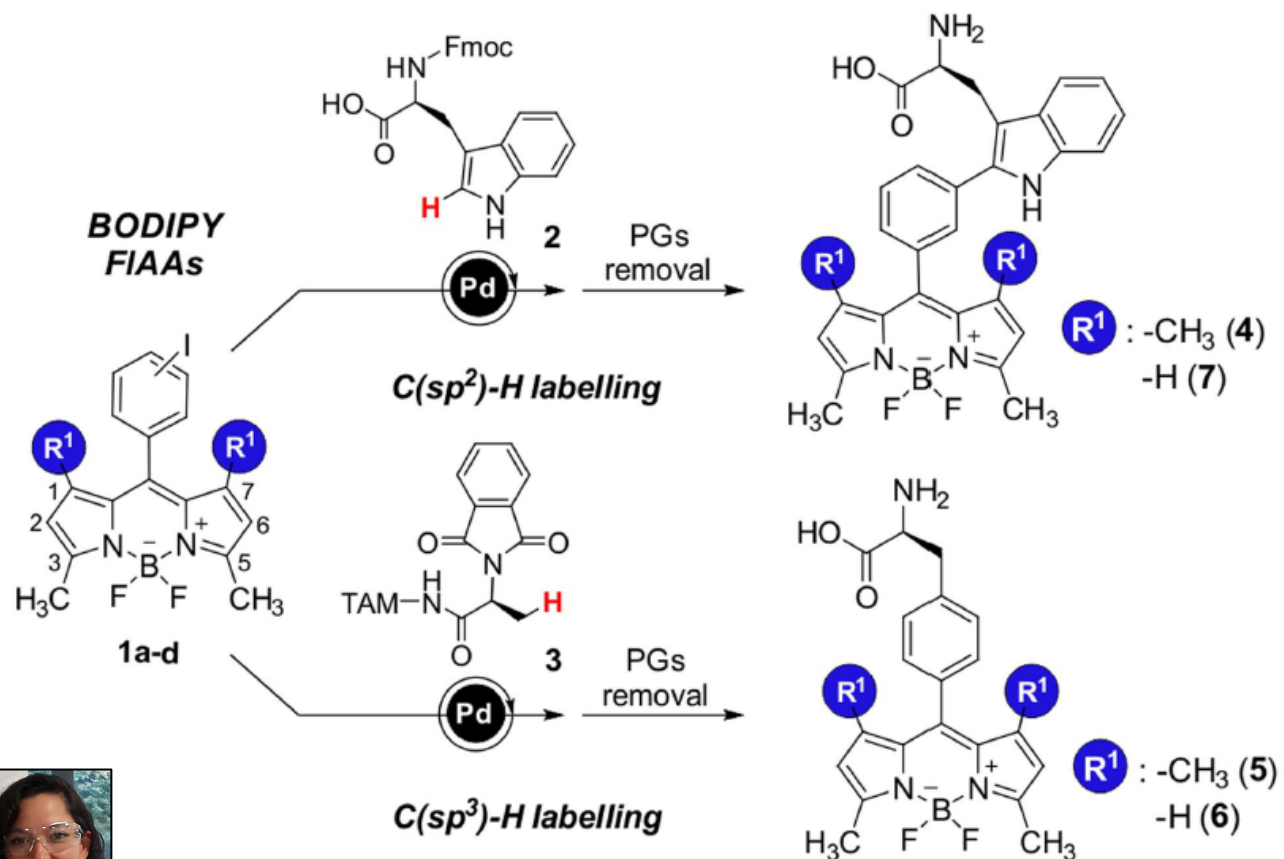


Can we make them smaller?



Fluorogenic (OFF-to-ON) amino acids

Smaller size (try 1: Phe-BODIPYs)

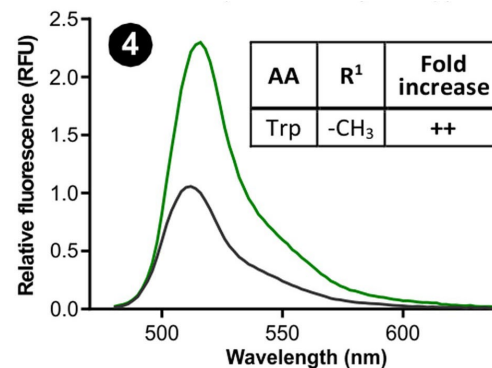
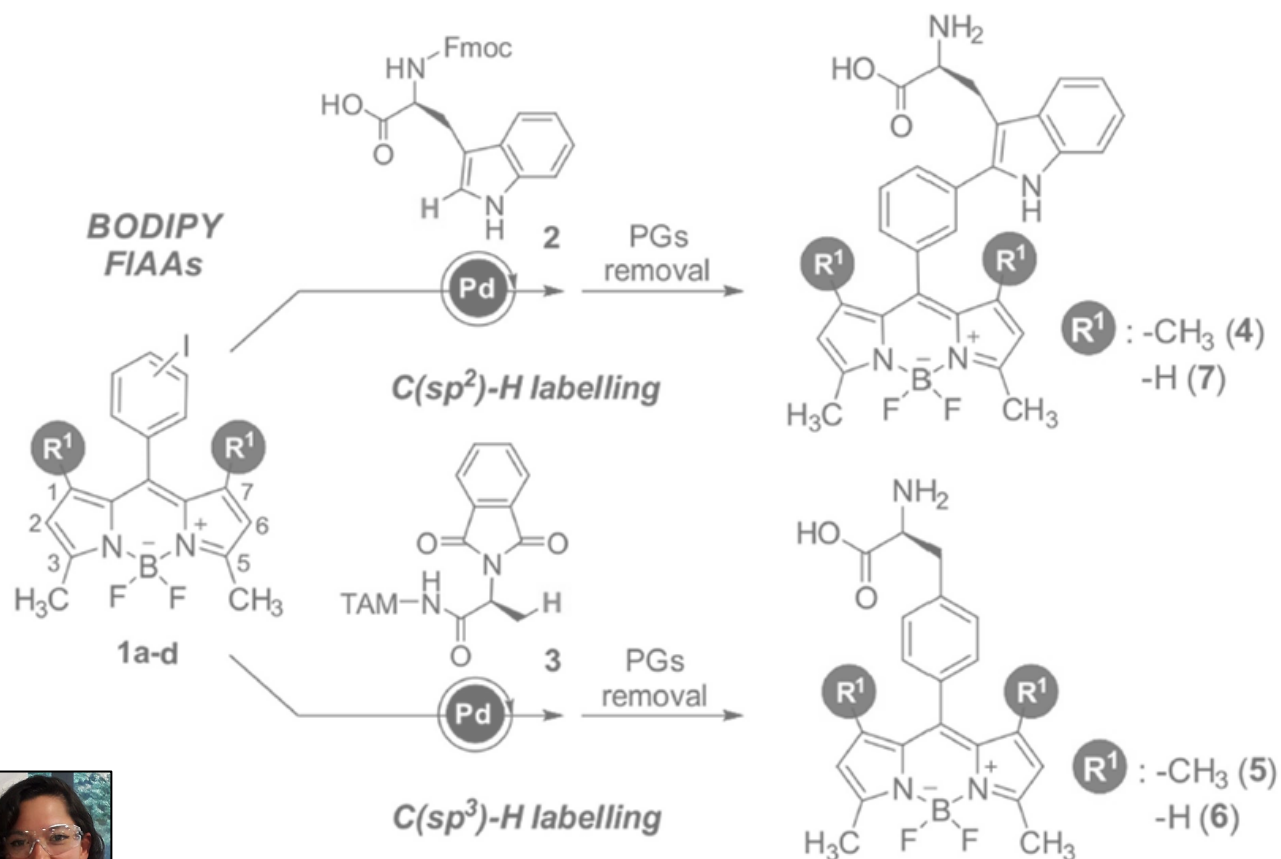


Mendive-Tapia et al. *Angew. Chem.*, 2022, e202302688.

Collaboration with Lutz Ackermann (previous work: Wang et al. *Angew. Chem.*, 2018, 14700).

Fluorogenic (OFF-to-ON) amino acids

Smaller size (try 1: Phe-BODIPYs)

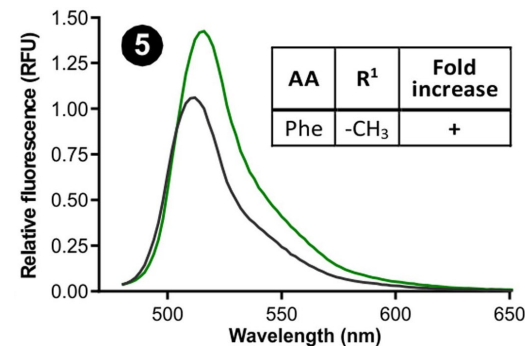


PeT

✓

rotation

X

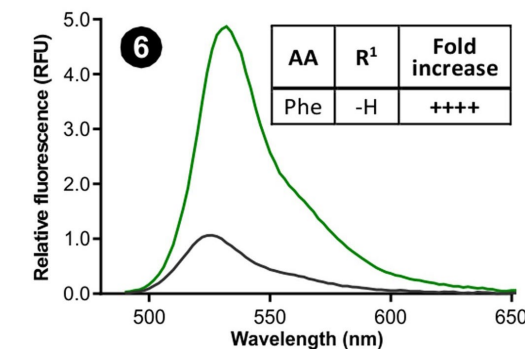


PeT

X

rotation

X



PeT

X

rotation

✓

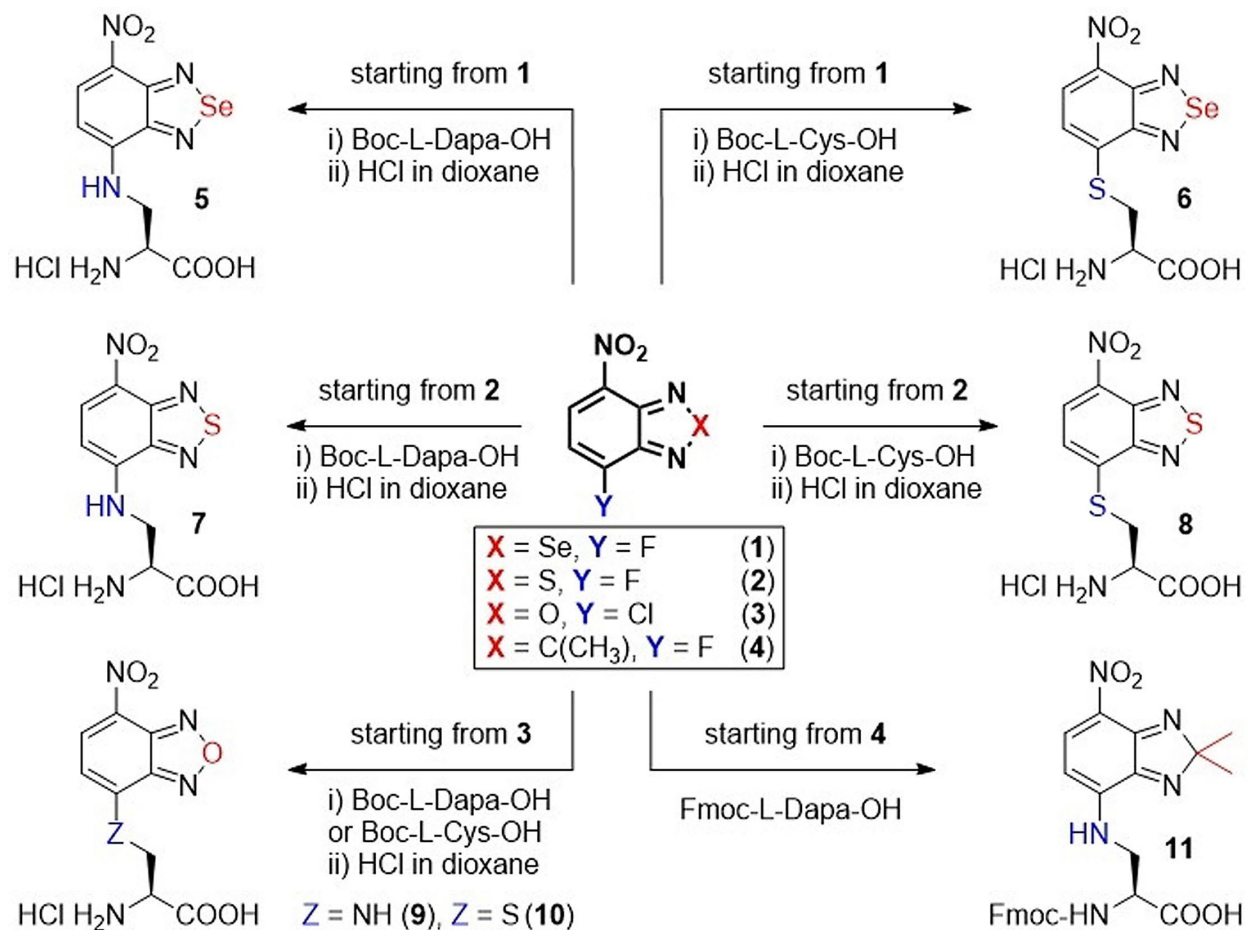


Mendive-Tapia et al. *Angew. Chem.*, 2022, e202302688.

Collaboration with Lutz Ackermann (previous work: Wang et al. *Angew. Chem.*, 2018, 14700).

Fluorogenic (OFF-to-ON) amino acids

Smaller size (try 2: NBDs)

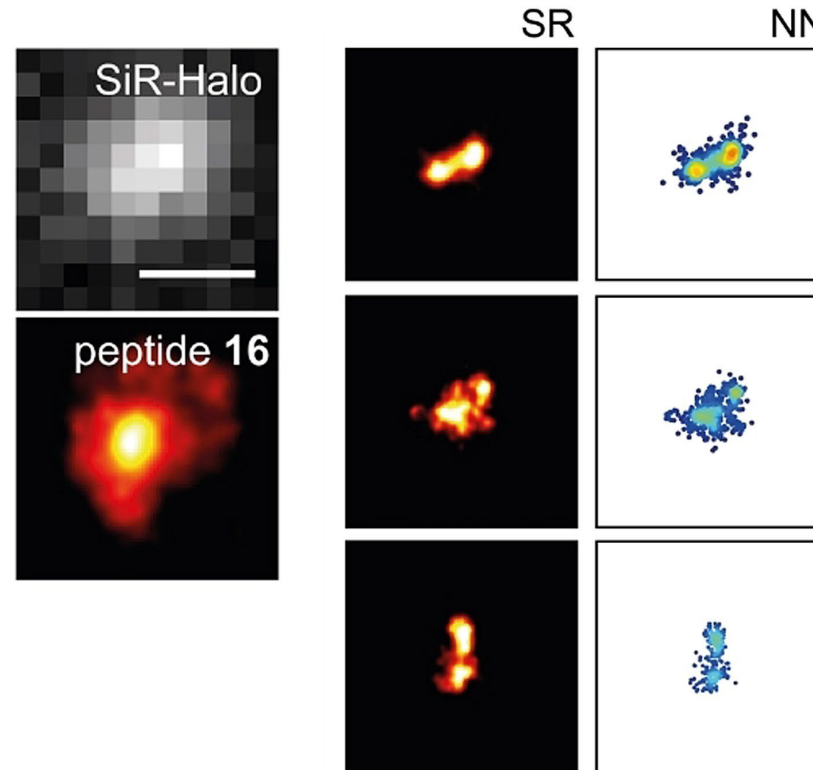
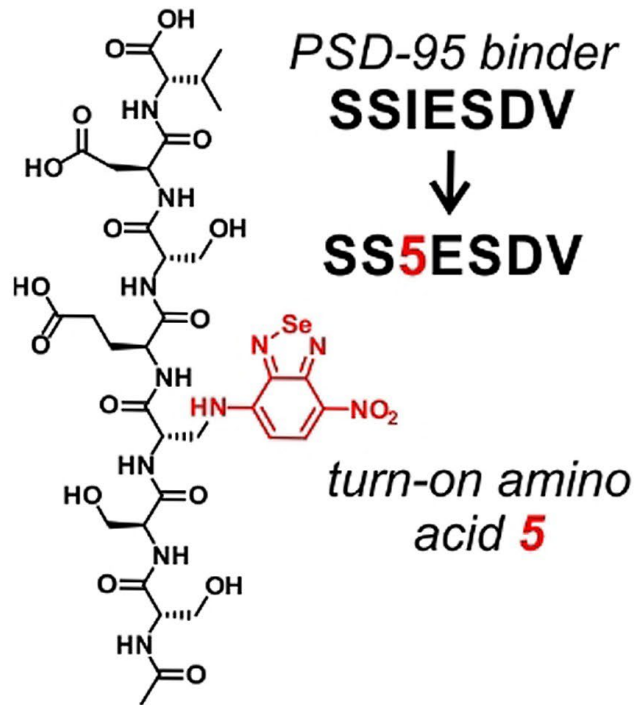


De Moliner and Konieczna et al. *Angew. Chem.*, 2023, e202216231.

Building on our previous work on SCOTfluors: Benson et al. *Angew. Chem.*, 2019, 6911; *Nat. Commun.*, 2021, 2369.

Fluorogenic (OFF-to-ON) amino acids

Smaller size (try 2: NBDs)



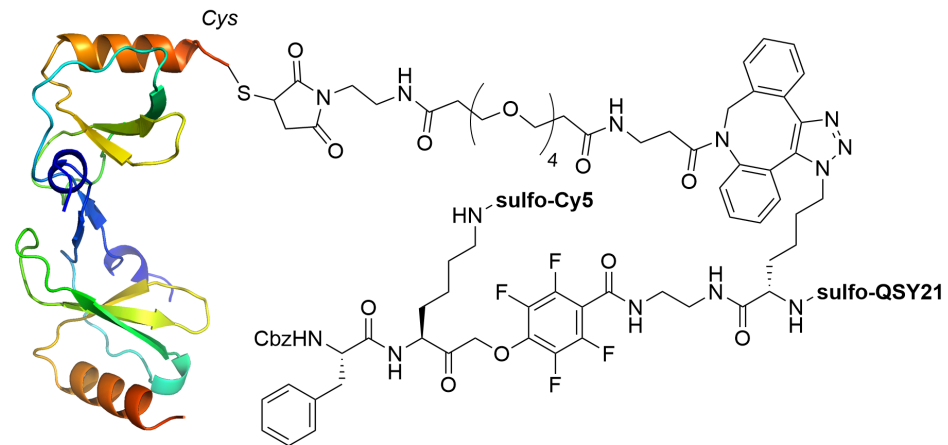
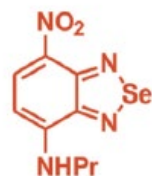
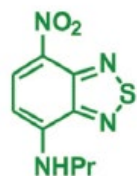
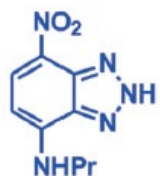
peptide PAINT imaging
of PSD-95 nanoclusters



De Moliner and Konieczna et al. *Angew. Chem.*, 2023, e202216231.

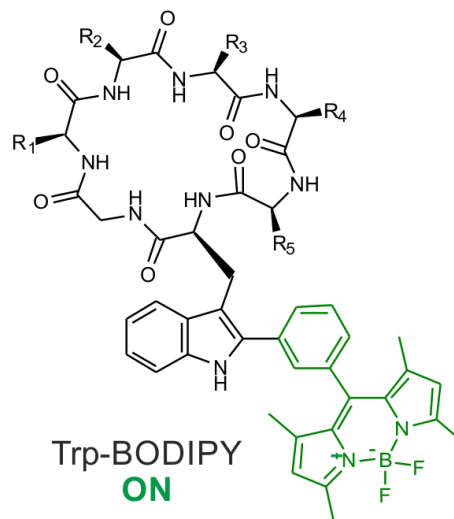
Collaboration with Mathew Horrocks for peptide-PAINT (previous work: Oi et al. *Commun. Biol.*, 2020, 458).

Imaging tool builders



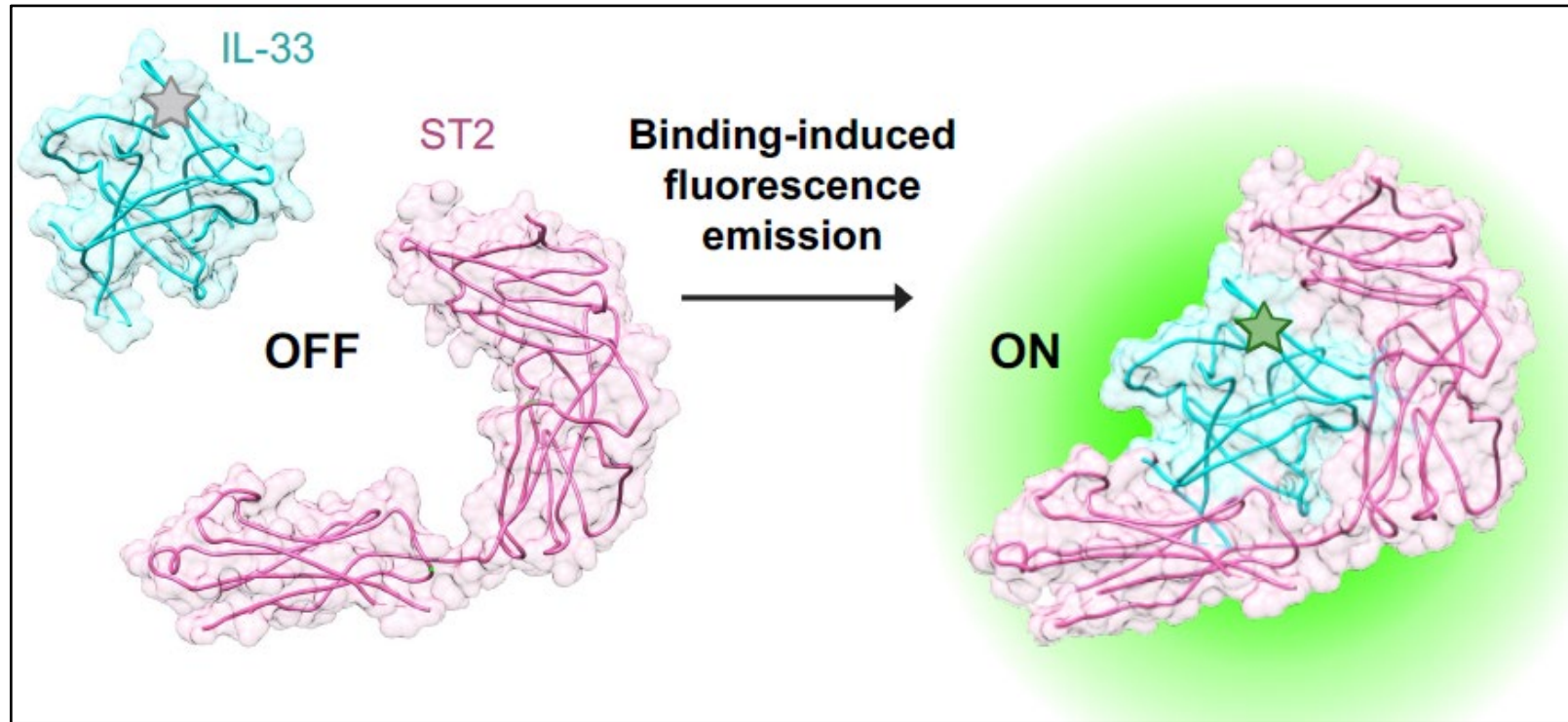
Fernandez et al. *Angew. Chem.*, 2019, 16894 (CCL2-PhagoGreen).
Barth et al. *Angew. Chem.*, 2022, e202207508 (CCL2-cathepsins).

molecular
weight



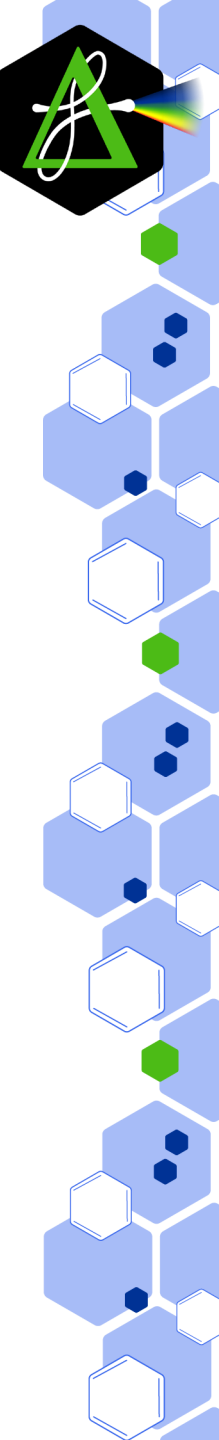
Fluorogenic proteins

Interleukin 33 (IL-33): alarmin cytokine involved in asthma, IBD...



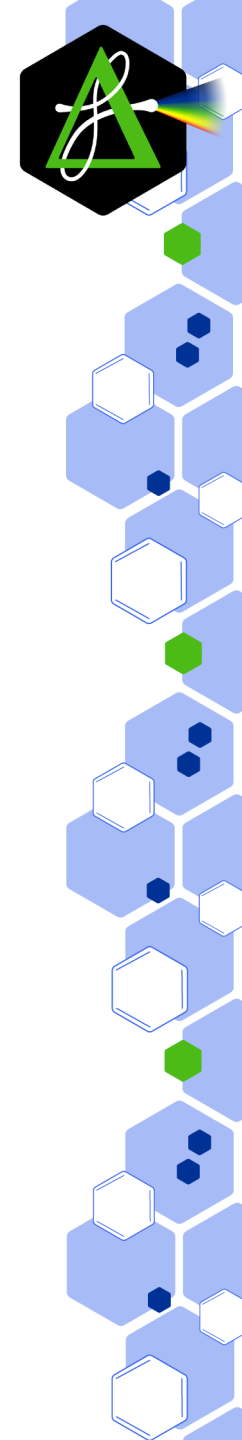
Reese et al. *ACS Cent. Sci.*, 2024, 143.

Collaboration with Henry McSorley (previous work on IL-33 biology: Osbourn et al. *Immunity*, 2017, 739).

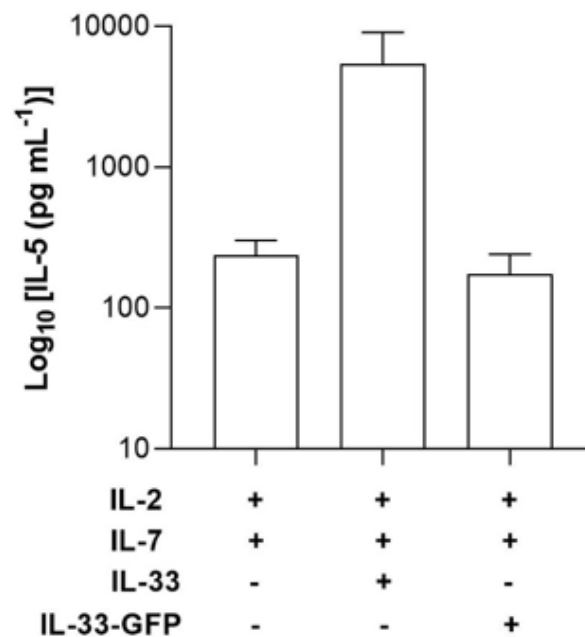
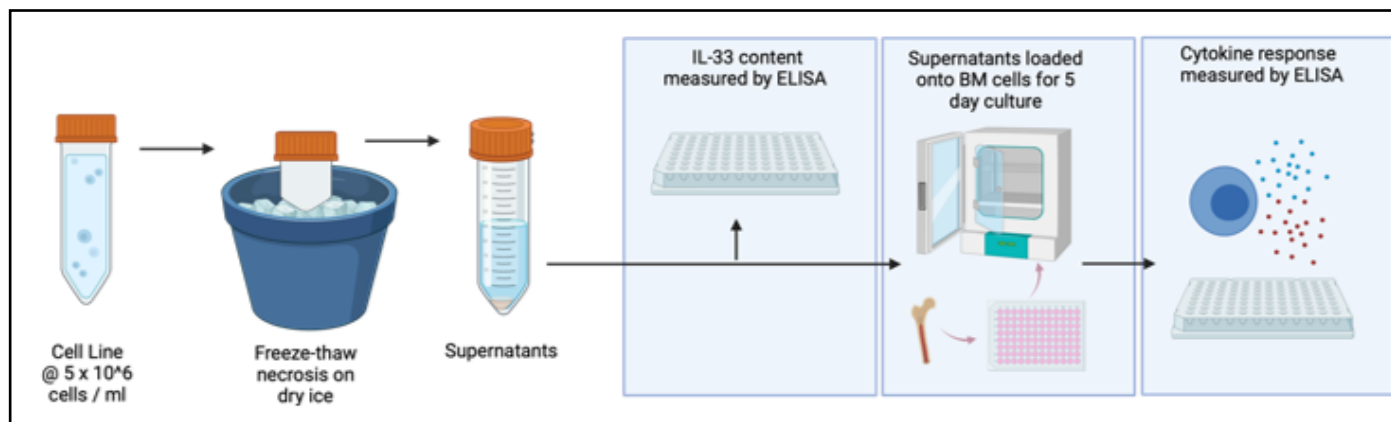


Fluorogenic proteins

*Can't we do this with GFP (**Green Fluorescent Protein**)?*



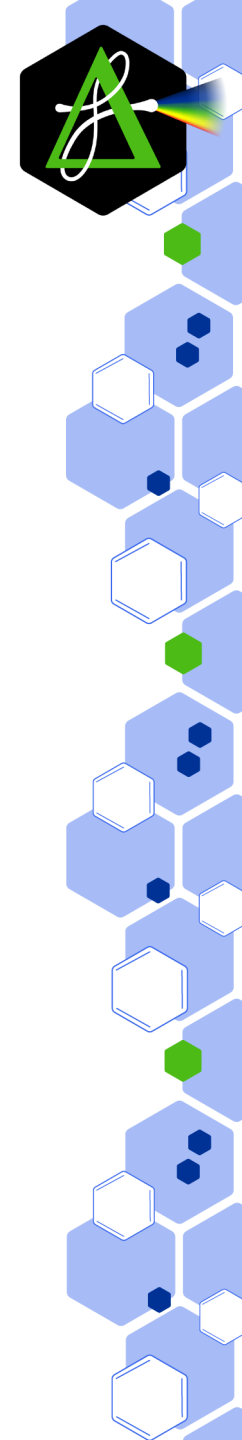
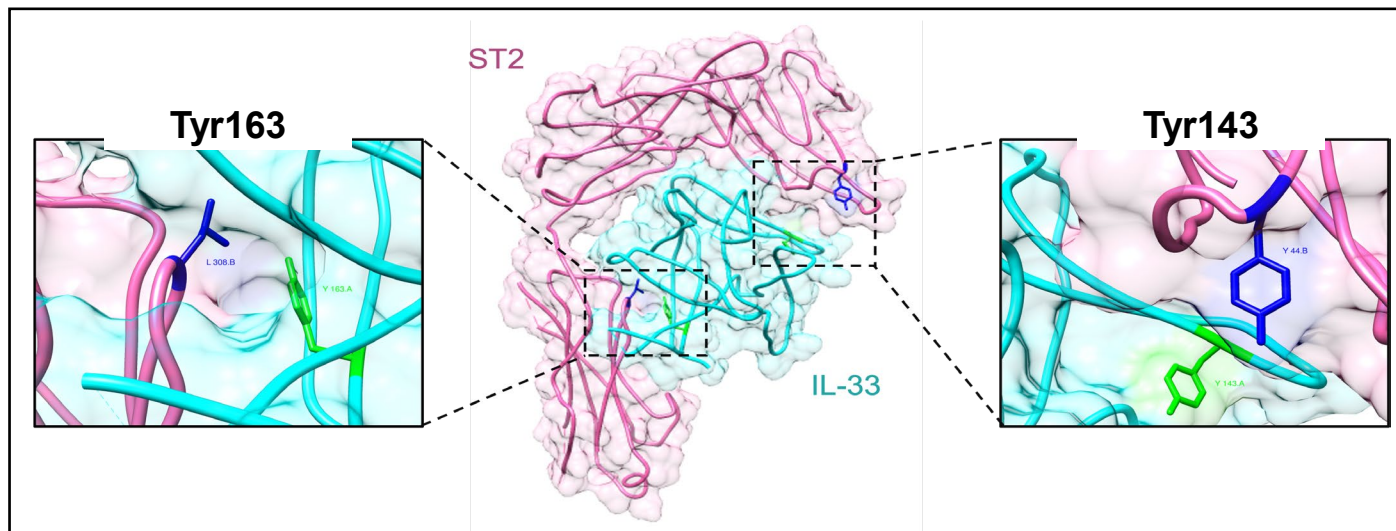
Fluorogenic proteins



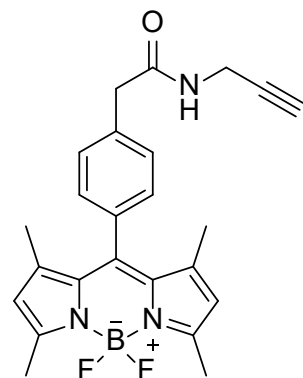
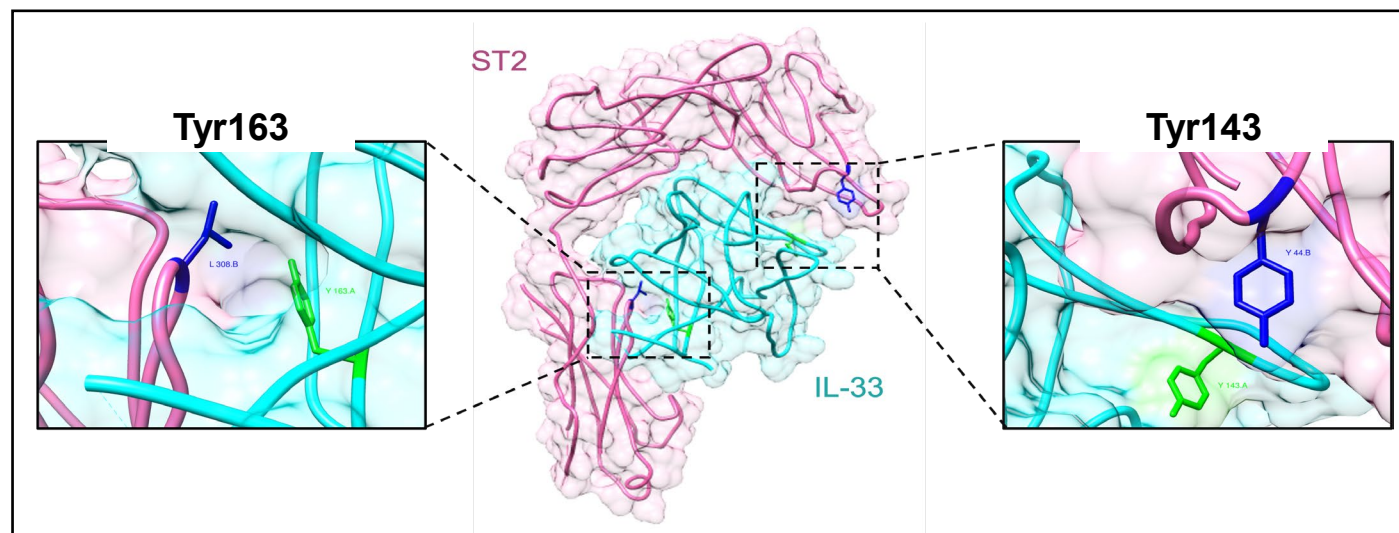
*No, we can't
(at least for IL-33)*



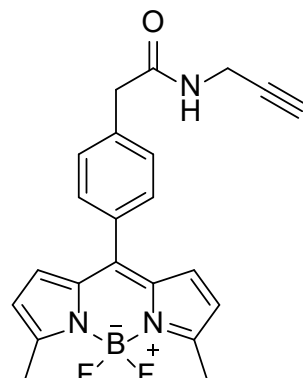
Fluorogenic proteins



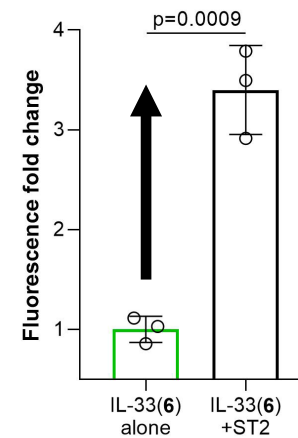
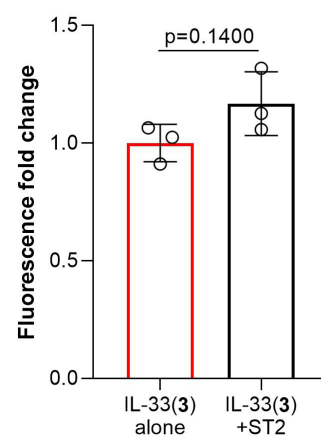
Fluorogenic proteins



always ON

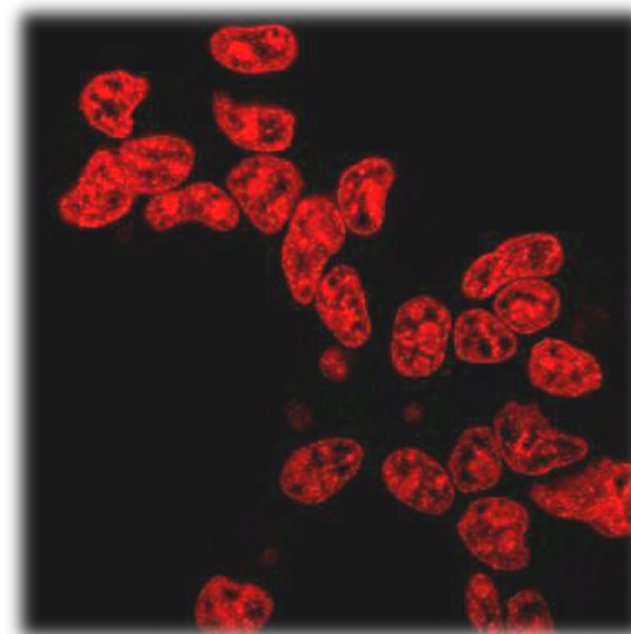
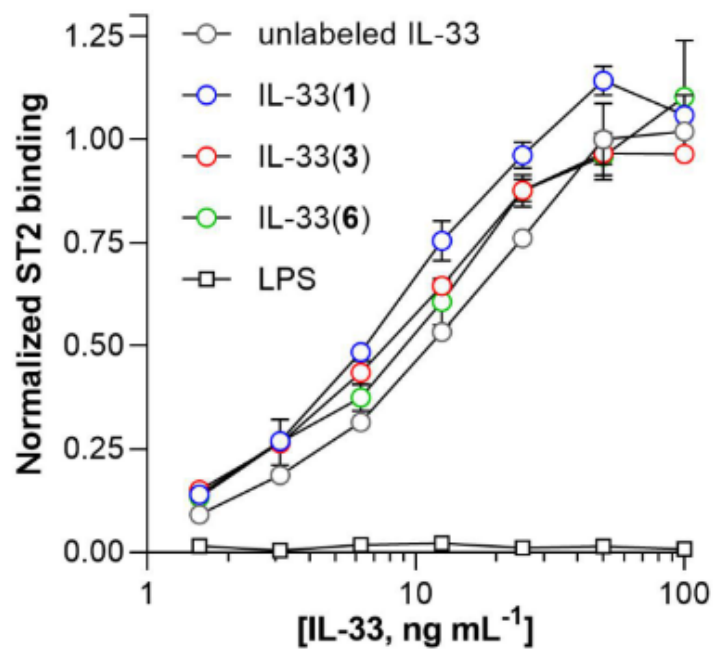


OFF-to-ON



Fluorogenic proteins

Native-like fluorogenic IL-33 proteins



real-time imaging of IL-33
in ST2+ve live cells (10× FF)

